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GROWTH INHIBITORS AND METHODS OF TREATING CANCER AND CELL PROLIFERATIVE DISEASES

Field of the Invention

This invention relates to the use of new and useful bioflavonoid compounds and related compounds which include methyl p-hydroxyphenyllactate (MeHPLA), its analogs, chemical derivatives and chemically related compounds, phenylmethylene ketones, nitroalkenes, aurones, and chalcones as antitumor agents, inhibitors of proliferative cell growth and immunosuppressive agents.

Background of the Invention

There are two types of nuclear estrogen binding sites in normal and malignant tissues. Type I sites represent the classical estrogen receptor and nuclear Type II sites appear to mediate a specific nuclear response to estrogenic hormones. After estrogen administration, Type I receptor sites bind estradiol and this receptor-estrogen complex interacts with nuclear acceptor sites before the initiation of the transcriptional events that are associated with estrogen stimulation of tissue growth. In contrast, Type II sites bind estrogen with a higher capacity and a lower affinity than the classical estrogen receptor and do not appear to be translocated from the cytoplasm to the nucleus. Thus, although the levels of nuclear Type II sites are increased by estrogen administration, Type II sites remain in the cytoplasm after hormone administration. Nuclear Type II sites appear to mediate a specific nuclear response to estrogenic hormones and are highly correlated with uterine cellular hypertrophy and hyperplasia. Additionally, nuclear Type II sites are highly stimulated in malignant tissues such as mouse mammary tumors and human breast cancer. This observation is consistent with the findings that highly proliferative tissue has an increased number of nuclear Type II sites. Because the stimulation of nuclear Type II sites is closely correlated with the stimulation of uterine growth, it has been postulated that the Type II sites are the location for the mechanisms by which estrogens cause uterotropic stimulation. Furthermore, the presence of Type II sites on the nuclear matrix suggests a potential role in the regulation of DNA synthesis.

Nuclear Type II sites are constituents of many, if not all, non-malignant cells. Normally, Type II sites in non-malignant cells are occupied by methyl p-hydroxyphenyllactate (MeHPLA or methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate). Methyl p-hydroxyphenyllactate (MeHPLA) is an endogenous ligand for nuclear type II binding sites in normal and malignant cells, as well as in lymphocyte cells of the immune system, and this compound regulates cell growth and proliferation through this binding interaction. MeHPLA may be derived endogenously from bioflavonoid (Griffiths and Smith (1972) Biochem, J., 128:901) and/or tyrosine metabolism (Karoum (1985) Biogenic Amines 2:269). Additionally, MeHPLA is metabolized by malignant cells, and the resulting deficiency of this compound in tumors is directly correlated with the loss of cell growth regulation. When MeHPLA binds to Type II sites, cell growth and proliferation of non-malignant tissues are slowed down or stopped. Conversely, malignant cells metabolize MeHPLA and, thus, there is insufficient binding to the nuclear Type II sites and the regulation of cell growth and proliferation is lost. Consequently, all tumor cell populations examined have very high levels of unbound nuclear Type II sites. This same metabolic activity is herein proposed as the probable mechanism wherein the compounds of the present invention regulate the cell proliferative activity in the immune system. These sites should represent targets for the analogs of MeHPLA as anti-proliferative agents.

This invention discloses compounds which are not metabolized by malignant or other rapidly proliferating cells such as those of the immune system but which bind to nuclear Type II sites with high affinity. These compounds are very effective inhibitors of tumor cell proliferation, DNA synthesis and lymphocyte activation. Therefore, the compounds of the present invention are also useful as immunosuppressive agents. Nuclear Type II sites have been observed in a variety of tumor and other proliferating cells such as those of the immune system. In addition, analogs and chemically related compounds such as phenylmethylene ketones, nitroalkenes, aurones, and chalcones are effective inhibitors of a broad spectrum of tumors and other rapidly proliferating cells such as activated lymphocytes. These compounds will be therapeutically effective in treating a wide variety of autoimmune diseases, as well as other pathological conditions of the immune system wherein inhibition of cell proliferation is desirable or necessary to treat the pathological condition. Consequently, any

tumor which contains nuclear Type II sites should respond to treatment with MeHPLA, its analogs, such as phenylmethylene ketones, nitroalkenes, aurones, and chalcones, derivatives and chemically related compounds, including cancers of the pancreas, cervix, liver, brain, pituitary, prostate and other organ or tissue sites, as well as other cancers, such as leukemias, lymphomas, stromal myomas and leiomyomas, among others. Since MeHPLA also blocks estrogen stimulation of normal cell growth such as that in the rat uterus (Table I), analogs and chemically related compounds of MeHPLA are also useful for the treatment of uterine hyperplasia, cervical hyperplasia, endometriosis and benign prostatic hypertrophy. Because non-proliferating non-malignant cells normally have their Type II sites bound with MeHPLA, the effects of the proposed compounds on non-malignant cell populations will be minimal to non-existent. For this reason, MeHPLA, its analogs and chemically related compounds, such as phenylmethylene ketones, nitroalkenes, aurones, and chalcones, derivatives and chemically related compounds and physiologically acceptable salts thereof are also useful as prophylactic agents in the inhibition and prevention of cancer, autoimmune disease, graft vs. host disease and abnormal proliferation of non-malignant cells.

The precise physiological role of Type II sites is unknown, but inhibition of the nuclear Type II sites is associated with antagonism of uterotropic responses to estrogen. This is true for steroid antagonists such as dexamethasone, progesterone and triphenylethylene derivatives such as nafoxidine and clomiphene. While there is at least one endogenous inhibitor of estradiol binding to nuclear Type II sites, no specific inhibitors for the nuclear Type II sites had been identified previous to those identified by some of the inventors of the present invention. Furthermore, the inhibitors of the present invention are specific to nuclear Type II sites and do not interfere with estradiol binding to cytoplasmic or nuclear Type I estrogen receptors.

The inhibitors are identified as methyl 3-(4- hydroxyphenyl)-2-hydroxypropionate, its analogs, derivatives and chemically related compounds, such as phenylmethylene ketones, nitroalkenes, aurones, and chalcones, and physiologically acceptable salts thereof and are potent regulators of cell growth and proliferation in normal and malignant tissues, as well as in the regulation of

immune function. One inhibitor is also known as methyl p-hydroxyphenyllactate or MeHPLA. These terms may be used interchangeably. Cell growth inhibition by these compounds resides in their ability to interact with the high-affinity nuclear binding sites in normal and malignant cells which may be involved in the regulation of cellular proliferation and DNA synthesis. When MeHPLA is bound to nuclear Type II sites, cell growth and proliferation are inhibited. The endogenous 3-(4-hydroxyphenyl)-2-hydroxypropionic acid inhibits the cell growth much less effectively. This activity correlates with its low binding affinity for nuclear Type II sites.

An additional object of the present invention is a means for the prevention of cancer. Since MeHPLA is a normal constituent of mammalian cells, but metabolized by malignant cells, MeHPLA, structural analogs and chemically related compounds as described herein, including, but not limited to phenylmethylene ketones, nitroalkenes, aurones, and chalcones, derivatives and chemically related compounds and physiologically acceptable salts thereof, which are not metabolized by tumors or other rapidly proliferating cells such as those of the immune system should be useful in the prevention of malignancy and in the treatment of many pathological conditions of the immune system, including, but not limited to autoimmune diseases. These compounds possess little if any side effects, and if taken in a low level maintenance dose should inhibit the proliferation of malignant cells, as well as lymphocytes of the immune system.

Because MeHPLA is such a potent inhibitor of cell growth, this compound, as well as its analogs and chemically related compounds were used as potential antitumor agents. The present invention discloses the potent antitumor and immunosuppressive activity of these compounds.

An object of the present invention is a treatment for cancer.

An additional object of the present invention is a procedure to inhibit the growth of proliferating cells which include a Type II nuclear estrogen binding site. A further object of the present invention is a method for inhibiting the growth of estrogen responsive tissues.

An additional object of the present invention is the treatment of human breast cancer, and other malignancies which contain unbound nuclear Type II sites.

Another object of the present invention is the treatment of benign prostatic hyperplasia, cervical hyperplasia, uterine hyperplasia and endometriosis.

An additional object of the present invention is to provide an immunosuppressive agent.

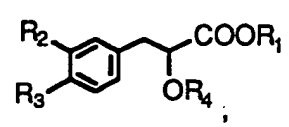
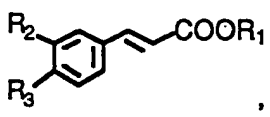
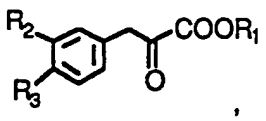
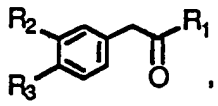
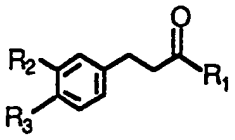
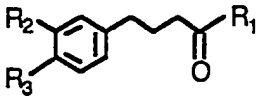
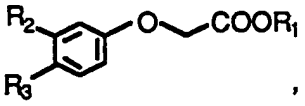
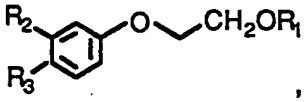
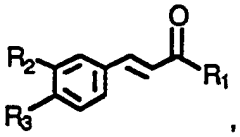
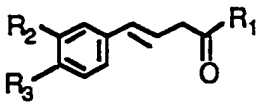
Summary of the Invention

Many anti-cancer drugs possess immunosuppressive- activity (Seldin and Steinberg (1988) In: "Inflammation Basic Principles and Clinical Correlates", (Galin, Goldstein, Snyderman, eds.) Raven Press, Ltd., New York). Many of the commonly used immunosuppressive agents were originally designed as anti-cancer drugs. Immunosuppressive drugs have proven to be therapeutically effective in treating a variety of autoimmune diseases.

An additional object of this invention is the treatment of autoimmune diseases, including, but not limited to, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, diabetes mellitis, thyroiditis, uveoretinitis, systemic lupus erythematosus, Sjorgen's syndrome, autoimmune skin diseases, and others.

An additional object of the present invention is to provide a treatment for graft vs. host disease and to prevent transplant rejections.

Thus, in accomplishing the foregoing objects, there is provided in accordance with one aspect of the present invention a method of treating cancer and autoimmune disease comprising the step of administering a therapeutic dose of MeHPLA, its analogs, chemical derivatives or chemically related compounds. More specifically the compound is selected from the group consisting of the formula: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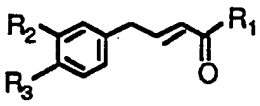
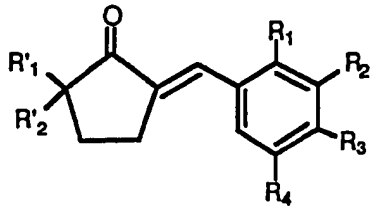
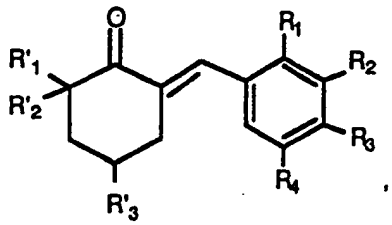
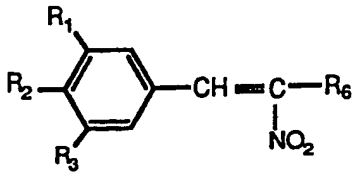


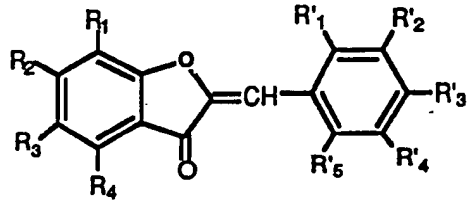
Wherein, R_1 is selected from the group consisting of H, alkyl groups containing 1 to 6 carbons, and aryl groups; R_2 and R_3 are selected from the group consisting of H, OH and OCH_3 and R_4 is selected from the group consisting of H, or alkyl group containing 1 to 6 carbons. Preferred compounds which may be used to practice the present invention may be selected from the group consisting of methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-propyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-butyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, 3-(4-hydroxyphenyl)-2-propenoic acid, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-3-pentanone, methyl (4-hydroxyphenoxy)acetate, and methyl 3-(3,4-dihydroxyphenyl)-2-propenoate.

Another aspect of the invention provides a method of treating cancer and pathological conditions of the immune system, including, but not limited to, autoimmune diseases and graft vs. host disease, comprising administering a therapeutic dose of a compound selected from the group consisting of the formulas:









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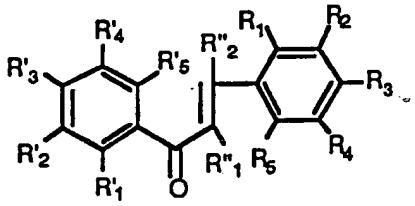
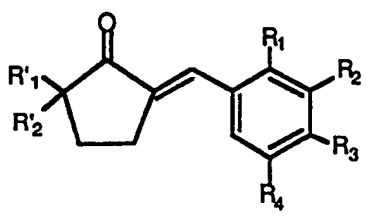


Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2 and R_3 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens and å-azido and aziridine derivatives, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens and å-azido and aziridine derivatives and halogen substituted derivatives. When the chalcone R''_1 and R''_2 is substituted to form an aziridine ring system, a three membered ring structure comprising the R''_1 and R''_2 carbons and a nitrogen atom is formed. Preferred compounds which may be used to practice the present invention may be selected from phenylmethylene ketones, nitroalkenes, aurones and chalcones.

Phenylmethylene ketones most preferred for practicing the present invention include



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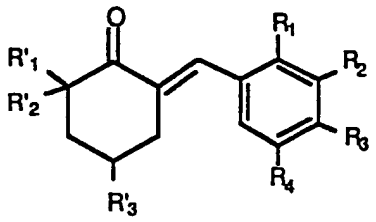
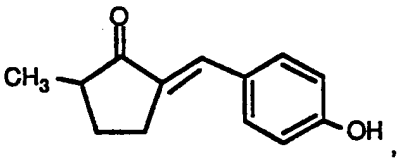


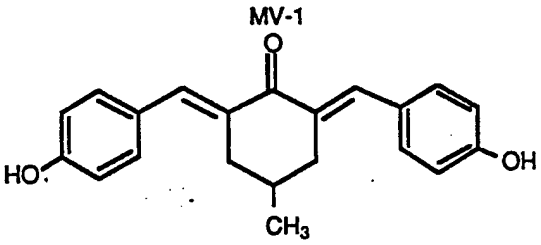
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

Most preferably the phenylmethylene ketones which may be used to practice the present invention are selected from the group consisting of



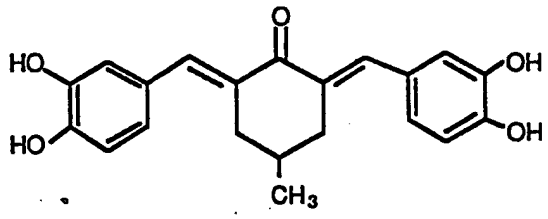
MV-1

MV-1



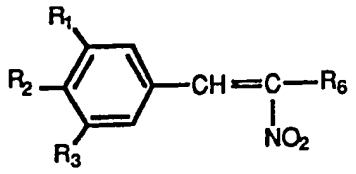
MV-17

and



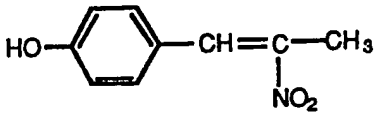
MV-18

Nitroalkenes most preferred for practicing the present invention include



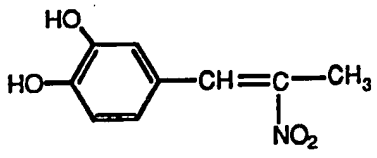
Wherein R_1, R_2 and R_3 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy and halogens, and R_6 is H, or alkyl group of 1 to 6 carbon atoms.

Among the nitroalkenes most preferably used to practice the present invention are:



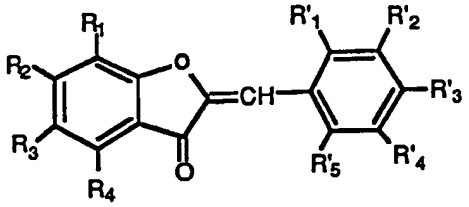
MV-N1

and



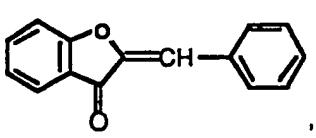
MV-N3

Among the aurones most preferable for practicing the present invention are:

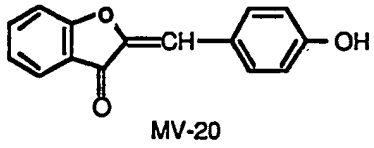


Wherein R_1-R_4 and R'_1-R'_5 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms.

The most preferable aurones useful in practicing the present invention are MV-19, MV-20 and MV-21.

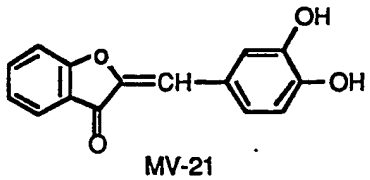


MV-19



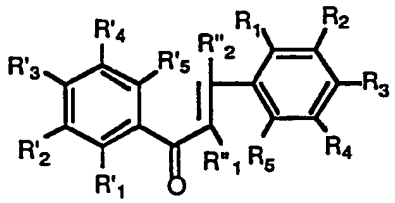
MV-20

and



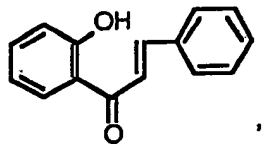
MV-21

Among the chalcones most preferable for practicing the present invention are:

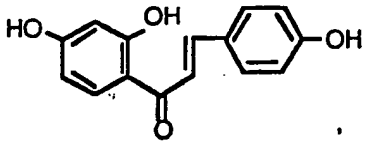


Wherein R_1-R_5, R'_1-R'_5, R''_1 and R''_2 are H, OH, OCH_3, amino,cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy and halogens and å-azido and aziridine derivatives.

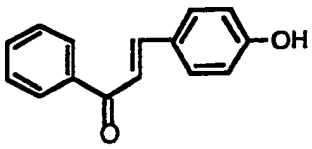
The most preferable chalcones useful in practicing the present invention are:



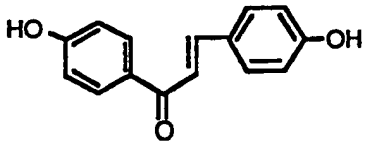
2'-hydroxychalcone



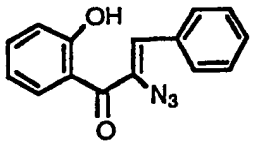
2',4',4-trihydroxychalcone



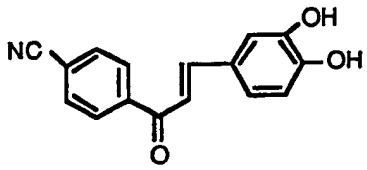
4-hydroxychalcone



4-4'-dihydroxychalcone

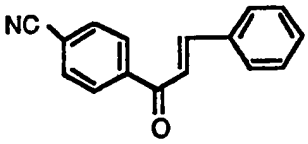


MV-35



MV-46

and



MV-47

Another aspect of the invention involves the inhibition of the growth of proliferating cells which include a Type II nuclear estrogen binding site by the step of administering a biological inhibiting dose of MeHPLA, its analogs, chemical derivatives or chemically related compounds such as phenylmethylene ketones, nitroalkenes, analogs and chalcones to the proliferating cells.

An additional aspect of the present invention is the inhibition of the proliferative growth of estrogen responsive tissues such as uterus, mammary gland, uterine tumors and mammary tumors. In one specific aspect, the above-

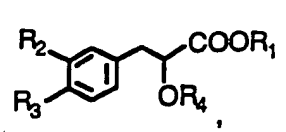
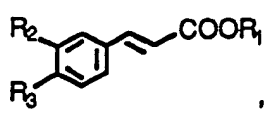
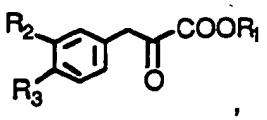
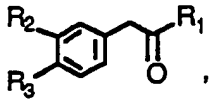
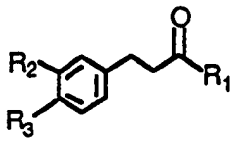
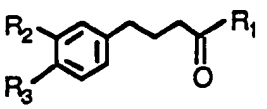
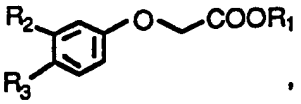
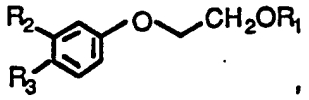
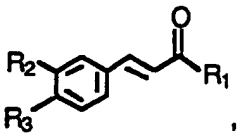
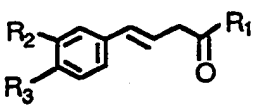
mentioned compounds have been used for the treatment of human breast cancer cells. The compounds inhibit the growth of human breast cancer cells.

An additional aspect of the present invention is the provision of an immunosuppressive agent. In one aspect these compounds are selected from the group consisting of MeHPLA, its analogs, derivatives and chemically related compounds including, but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, and physiologically acceptable salts thereof.

Another specific aspect of the present invention is a method for treating benign prostatic hyperplasia comprising the step of administering a therapeutic dose of MeHPLA, its analogs, derivatives and chemically related compounds including, but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, and physiologically acceptable salts thereof.

Another specific aspect of the present invention is a method for treating autoimmune diseases, including, but not limited to, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, diabetes mellitis, thyroiditis, uveoretinitis, systemic lupus erythematosus, Sjogren's syndrome, autoimmune skin diseases, and others comprising the step of administering a therapeutic dose of MeHPLA, its analogs, derivatives and chemically related compounds including, but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, and physiologically acceptable salts thereof.

Another specific aspect of the present invention is a method for treating autoimmune diseases, including, but not limited to, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, diabetes mellitis, thyroiditis, uveoretinitis, systemic lupus erythematosus, Sjogren's syndrome, autoimmune skin diseases, and others comprising the step of administering a therapeutic dose of a compound selected from the group consisting of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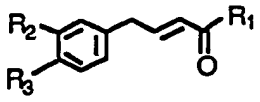
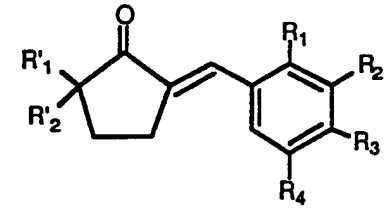
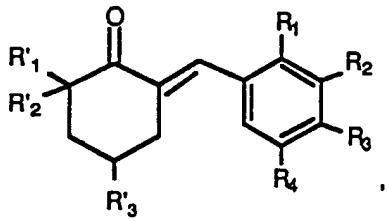
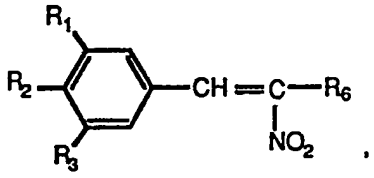


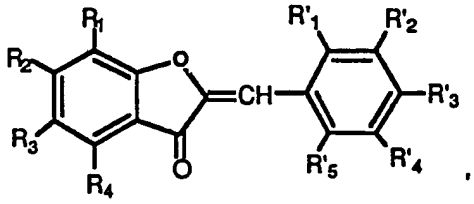
Wherein, R_1 is selected from the group consisting of H, alkyl groups containing 1 to 6 carbons, and aryl groups; R_2 and R_3 are selected from the group consisting of H, OH and OCH_3 and R_4 is selected from the group consisting of H, or alkyl group containing 1 to 6 carbons. Preferred compounds which may be used to practice the present invention may be selected from the group consisting of methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-propyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-butyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, 3-(4-hydroxyphenyl)-2-propenoic acid, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-3-pentanone, methyl (4-hydroxyphenoxy)acetate, and methyl 3-(3,4-dihydroxyphenyl)-2-propenoate.

and









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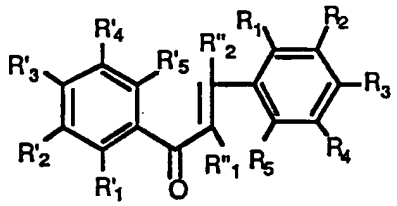


Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, CH_3 and OCH_3, acyloxy and halogen substituted derivatives. Preferred compounds which may be used to practice the present invention may be selected from phenylmethylene ketones, nitroalkenes, aurones and chalcones.

Another aspect of the present invention is the provision of an antitumor agent which comprises analogs of MeHPLA, including, but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, derivatives and chemically related compounds and physiologically acceptable salts thereof.

Another aspect of the present invention is the provision of an immunosuppressive agent which comprises analogs of MeHPLA, including, but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, derivatives and chemically related compounds and physiologically acceptable salts thereof.

Another aspect of the present invention is prophylactic agents to inhibit and prevent cancer, autoimmune disease, graft versus host disease and non-malignant cell growth. These prophylactic agents include the above-mentioned MeHPLA, its analogs, including but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, chemical derivatives or chemically related compounds and pharmaceutically acceptable salts thereof.

Other and further objects, features and advantages will be apparent from the following description of the presently preferred embodiments of the invention given for the purpose of disclosure.

Brief Description of the Figures

Figure 1 represents the competition of MV-3, MV-12 and MV-88 for [^{3}H]estradiol binding to nuclear Type II sites.

Figure 2 represents the analysis of nuclear type II binding sites in popliteal Lymph node nuclei.

Figure 3 represents the effects of MV-3, MV-12, and MV-88 on MCF-7 human breast cancer cell proliferation.

Figure 4 represents the effects of the compounds of the present invention on Mouse Mammary tumor growth in vivo.

Figure 5 represents the effects of MV-19, MV-20 and MV-21 on mouse mammary tumor growth in vivo.

Figure 6 demonstrates the effects of cyclodextrin encapsulated MV-88 on mouse mammary tumor growth in vivo.

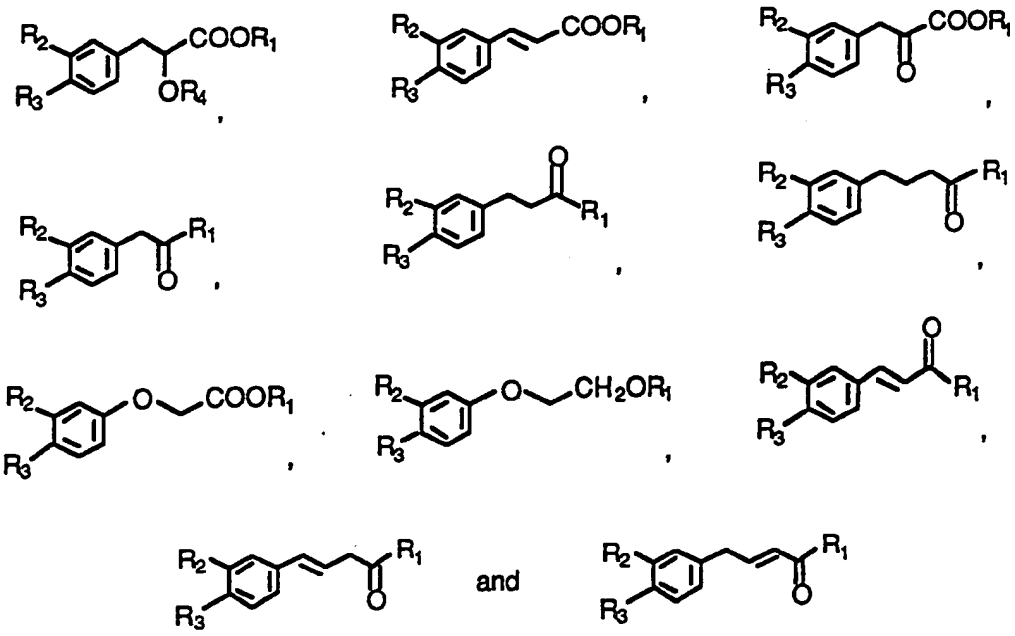
Figure 7 demonstrates the inhibition of the development of autoimmune uveoretinitis.

Detailed Description of the Specific Embodiment

Definitions.

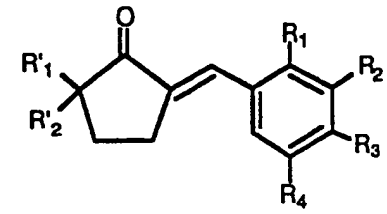
"Methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate" is also known as methyl p-hydroxyphenyllactate or MeHPLA. The term "MeHPLA" is meant to also include its analogs, chemical derivatives, and chemically related compounds which bind to the nuclear Type II receptors and by so doing inhibit cell proliferation.

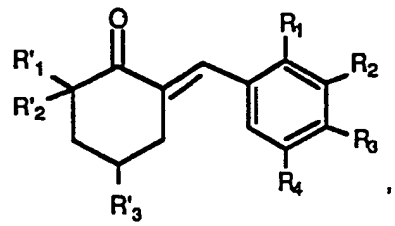
The term "chemically related compounds" refers to the derivatives and analogs of p-coumaric acid, p-hydroxyphenylbutanone, (4-hydroxyphenoxy)acetate and the arylpropenaldehydes, alkyl arylethenyl ketones, aryl arylethenyl ketones, aryl butenaldehydes, alkyl arylpropenyl ketones and arylpropenyl ketones which are structurally related to MeHPLA and disclosed herein. These chemically related compounds include the cis and trans isomers of said compounds and their esters, ethers, ketones and derivatives containing S or N in place of O atoms. More specifically these structurally related analogs and derivatives include compounds where R_1 represents the methyl, ethyl, n-propyl, n-butyl, isopropyl, tert-butyl or aryl group and R_2 and R_3 represent H, OH or OCH_3 groups and R_4 is H or an alkyl group of 1 to 6 carbons. Specific analogs of each class of these structurally related compounds to MeHPLA have been demonstrated to possess biological activity (Tables I and II) as defined herein and therefore mimic MeHPLA as an effective inhibitor of cell proliferation, tumor cell growth and as immunosuppressive agents. Preferably, the analogs and chemically related compounds effective in practicing the present invention include, but are not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones. Most preferably, these analogs are selected from the group consisting of the general formulas:

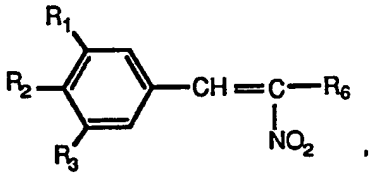


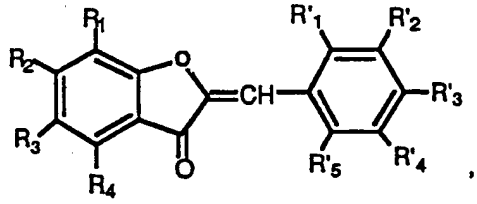
Wherein, R_1 is selected from the group consisting of H, alkyl groups containing 1 to 6 carbons, and aryl groups; R_2 and R_3 are selected from the group consisting of H, OH and OCH_3 and R_4 is selected from the group consisting of H, or alkyl group containing 1 to 6 carbons. Preferred compounds which may be used to practice the present invention may be selected from the group consisting of methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-propyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-butyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, 3-(4-hydroxyphenyl)-2-propenoic acid, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-3-pentanone, methyl (4-hydroxyphenoxy)acetate, and methyl 3-(3,4-dihydroxyphenyl)-2-propenoate.

Another aspect of the invention provides a method of treating cancer and pathological conditions of the immune system, including, but not limited to, autoimmune diseases and graft vs. host disease, comprising administering a therapeutic dose of a compound selected from the group consisting of the formulas:









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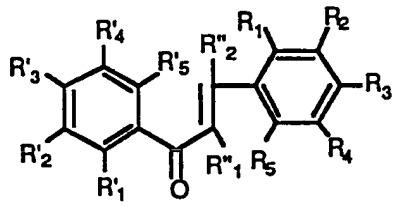
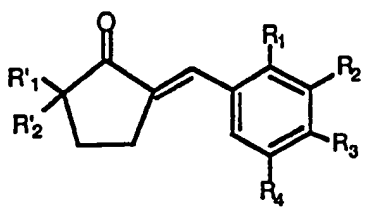


Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2 and R_3 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens and å-azido and aziridine derivatives, acyloxy and halogen substituted derivatives. Preferred compounds which may be used to practice the present invention may be selected from phenylmethylene ketones, nitroalkenes, aurones and chalcones.

Phenylmethylene ketones most preferred for practicing the present invention include



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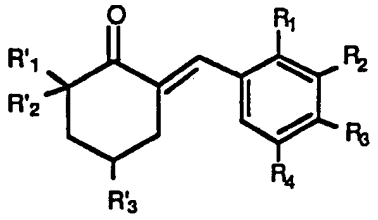
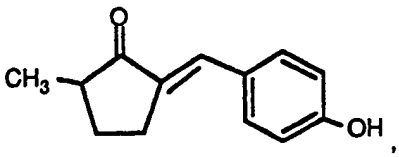


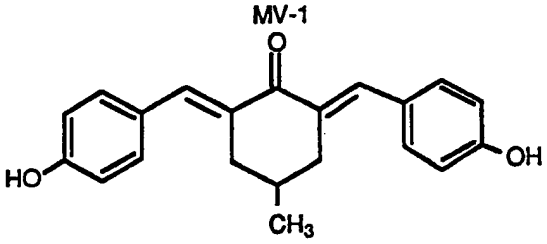
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

Most preferably the phenylmethylene ketones which may be used to practice the present invention are selected from the group consisting of



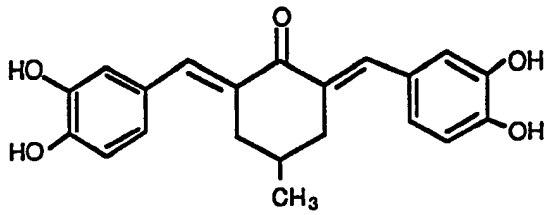
MV-1

MV-1



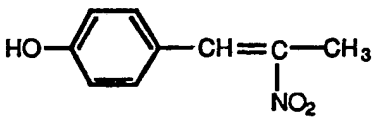
MV-17

and



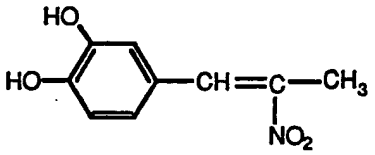
MV-18

Among the nitroalkenes most preferably used to practice the present invention are:



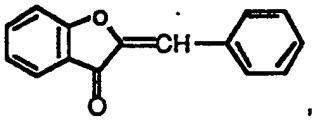
MV-N1

and

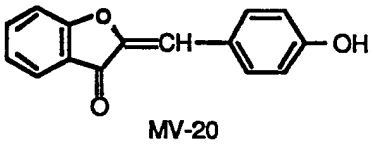


MV-N3

Among the aurones most preferable for practicing the present invention are:

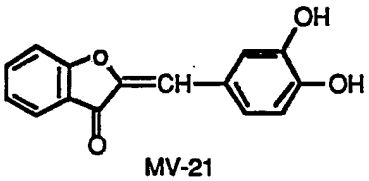


MV-19



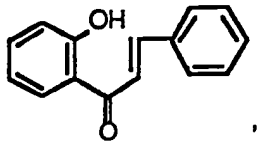
MV-20

and

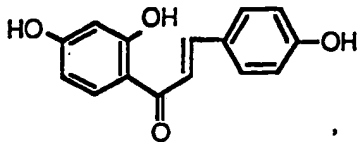


MV-21

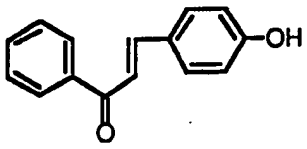
Among the chalcones most preferable for practicing the present invention are:



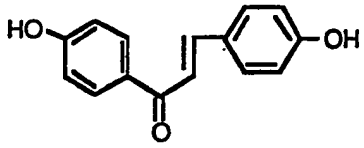
2'-hydroxychalcone



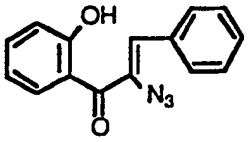
2',4',4-trihydroxychalcone



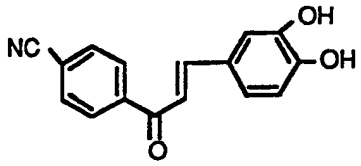
4-hydroxychalcone



4-4'-dihydroxychalcone



MV-35



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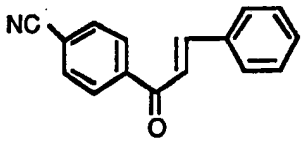


MV-47

The term "individual" is meant to include animals and humans.

The term "biologically inhibiting" or "inhibition" of the growth of proliferating cells is meant to include partial or total growth inhibition and also is meant to include decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose of the compounds of the present invention may be determined by assessing the effects of the test compound on malignant cell growth in tissue culture (see Figure 3), uterine growth in the animal (see Figures 14 and

15) or tumor growth in the animal as previously described by Markaverich et al., Cancer Research 43:3208-3211 (1983), or any other method known to those of ordinary skill in the art. These methods have also been fully described in U.S. Patent Application No. 219,680 which is incorporated herein by reference as if it appeared in full.

The term "immunosuppresive" or "immunosuppressing" or "suppression of the immune system" is meant to include partial or total immune suppression and is also meant to include changes in immune function such that "abnormal" immune functions become more normalized. The immunosuppressive dose of the compounds of the present invention may be determined by assessing the effects of the test compound in the established rat model of experimental autoimmune uveoretinitis as described by Gery et al., (1986) Invest. Ophthalmol. Vis. Sci. 27:1296, or any other method known to those of ordinary skill in the art.

The term "prophylactic agent" is meant to include agents which may be used for partial or total inhibition or prevention of disease and the spread of disease and also is meant to include agents which may be used as a precaution against disease and for preventive treatment of disease.

Administration of the compounds useful in the method of the present invention may be by topical, parenteral, oral, intranasal, intravenous, intramuscular, subcutaneous, or any other suitable means. The dosage administered is dependent upon the age, weight, kind of concurrent treatment, if any, and nature of the malignancy or the pathological immune condition. The effective compound useful in the method of the present invention may be employed in such forms as capsules, tablets, liquid solutions, suspensions, or elixirs, for oral administration, or sterile liquid forms such as solutions, suspensions or emulsions. Any inert carrier is preferably used, such as saline, or phosphate-buffered saline, or any such carrier in which the compounds used in the method of the present invention have suitable solubility properties.

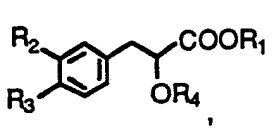
The compounds of the present invention may be administered in a biologically effective carrier. The biologically effective carriers may include any

solvent with which the compounds of the present invention are compatible and which are non-toxic to the individuals treated at the amounts administered.

Most preferably, the compounds of the present invention are administered as an encapsulated composition. Due to the low aqueous solubility of many of the compounds effective in carrying out the present invention, another aspect of the present invention comprises the drug delivery system of the compounds of the present invention encapsulated in cyclodextrin, liposomes or as silastic implants. However, the compounds of the present invention may be encapsulated by other methods and with other compounds by methods known to those skilled in the art.

The term "antitumor agent" is meant to include agents which decrease cell growth, or inhibit the proliferation of tumor cells when administered to said tumor cells in an effective dose.

One specific embodiment of this invention is an antitumor agent including MeHPLA, its analogs, chemical derivatives or chemically related compounds. Specific examples of MeHPLA analogs are derivatives of the general formula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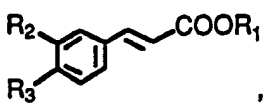
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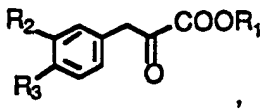
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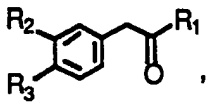
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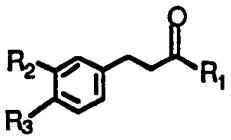
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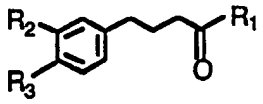
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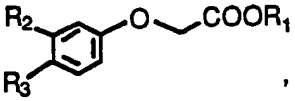
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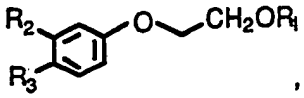
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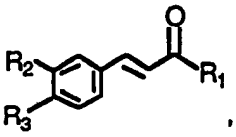
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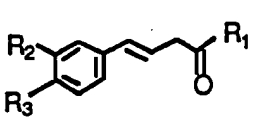
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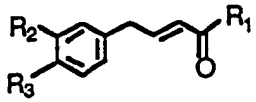
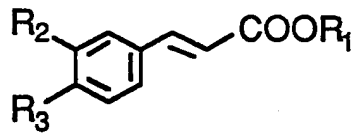


Wherein, R_1 is selected from the group consisting of H, alkyl groups containing 1 to 6 carbons, and aryl groups; R_2 and R_3 are selected from the group consisting of H, OH and OCH_3 and R_4 is selected from the group consisting of H, or alkyl group containing 1 to 6 carbons. Preferred compounds which may be used to practice the present invention may be selected from the group consisting of methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-propyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-butyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, 3-(4-hydroxyphenyl)-2-propenoic acid, 4-(4-hydroxyphenyl)-2-butanone, 1-(4-hydroxyphenyl)-3-pentanone, methyl (4-hydroxyphenoxy)acetate, and methyl 3-(3,4-dihydroxyphenyl)-2-propenoate.

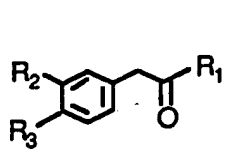
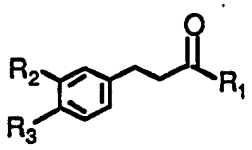
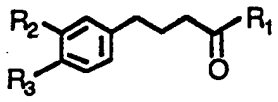
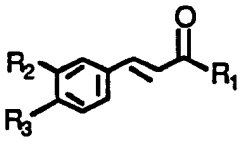
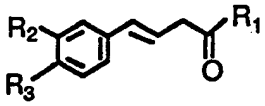
One specific example of this type of compound is wherein R_1 is CH_3, R_2 is H, R_3 is OH and R_4 is H. This is a naturally occurring endogenous compound which was isolated and characterized and identified as the present invention. Other examples of analogs include the compounds in which the R group has been replaced by an ethyl, n-propyl, n-butyl, isopropyl, tert-butyl or aryl group; R_2 and/or R_3 have been replaced with an H, OH or OCH_3 group and R_4 is H or an alkyl group of 1 to 6 carbons. Each of these esters can exist in the D and L form.

Another group of derivative compounds includes those with the formula:



Examples of these compounds are p-coumaric acid, 3-(4-hydroxyphenyl)-2-propenoic acid, and its esters. These substances exist as cis and trans isomers. In coumaric acid R_1 and R_2 are hydrogen and R_3 is OH. Additional esters include compounds wherein R_1 is methyl, ethyl, n-propyl, n-butyl, isopropyl, tert-butyl or aryl and R_2 and/or R_3 is a H, OH or OCH_3 group. Additional analogs include caffeic acid, 3-(3,4-dihydroxyphenyl)-2-propenoic acid, wherein R_1 is H and R_2 and R_3 are both OH.

Other compounds with antitumor activity are the derivatives of l-(4-hydroxyphenyl)-3-butanone, such as compounds with the formula: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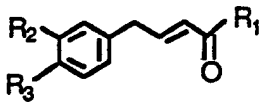
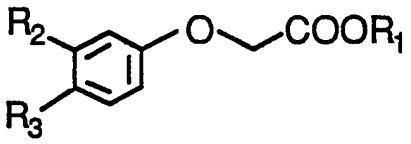


Wherein, R1 is selected from the group consisting of H, alkyl groups containing l to 6 carbons, and aryl groups; R2 and R3 are selected from the group consisting of H, OH and OCH3 and R4 is selected from the group consisting of H, or alkyl group containing l to 6 carbons. Preferred compounds which may be used to practice the present invention may be selected from the group consisting of methyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, n-propyl 3-(4-hydroxyphenyl)-2-hydroxypropionate**, n-butyl 3-(4-hydroxyphenyl)-2-hydroxypropionate, 3-(4-hydroxyphenyl)-2-propenoic acid, 4-(4-hydroxyphenyl)-2-butanone, l-(4-hydroxyphenyl)-3-pentanone, methyl (4-hydroxyphenoxy)acetate, and methyl 3-(3,4-dihydroxyphenyl)-2-propenoate.

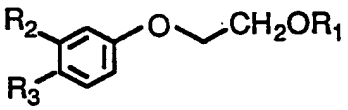
Ketone derivatives include compounds with a methyl, ethyl, n-propyl, n-butyl, isopropyl, tert-butyl or aryl group at the R_1 position, H, OH or OCH_3 group at the R_2 and R_3 positions; and most preferably H at the R_2 position and OH at the R_3 position.

Additionally, as can be seen by the formulae, the number of CH_2 groups between the aromatic entity and the keto group can be varied. Specific examples of compounds are l-(4-hydroxyphenyl)-3-pentanone and l-(4-hydroxyphenyl)-3-butanone. These compounds have been shown to bind to Type II sites and to have antitumor and anti-proliferative activity in the uterotropic assay.

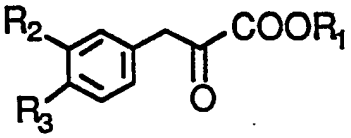
Another group of compounds which show anti-proliferative activity in the rat uterus is described by the formula:



These compounds are ether-linked analogs of (4-hydroxyphenoxy)acetic acid, for example, methyl (4-hydroxyphenoxy)acetate. All of these compounds bind to the Type II binding sites. These ether-linked compounds include analogs wherein R_1 is H, a C_1 to C_6 alkyl carbon chain or an aryl group, R_2 and R_3 are H, OH or OCH_3. An additional variation on the phenoxy compounds include the ether compounds, for example, 2-(4-hydroxyphenoxy) ethyl methyl ether, wherein R_1 can be H or any C_1 to C_6 alkyl carbon chain or an aryl group, R_2 and R_3 are H, OH or OCH_3 in the formula:

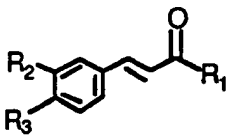


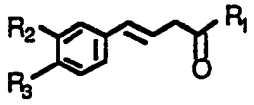
Additionally useful is the compound of the formula:



Wherein R_1 is a C_1 to C_6 alkyl chain or an aryl group, R_2 and R_3 are H, OH or OCH_3.

Another group of compounds which show tumor anti-proliferative action is described by the general formulas:





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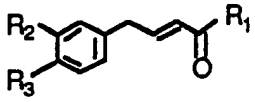
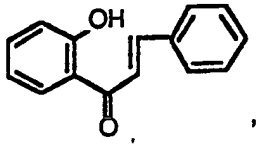
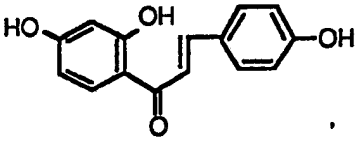


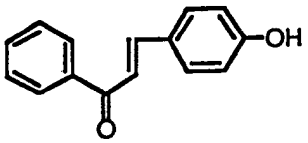
Wherein R_1 is from the group consisting of H, alkyl groups containing 1 to 6 carbons, and substituted or unsubstituted aryl groups; and R_2 and R_3 are selected from the group consisting of H, OH and OCH_3. Preferred compounds of this group which may be used to practice the present invention are:



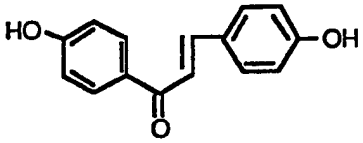
2'-hydroxychalcone



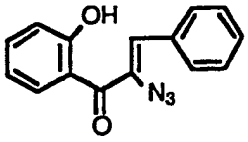
2',4',4-trihydroxychalcone



4-hydroxychalcone



4-4'-dihydroxychalcone



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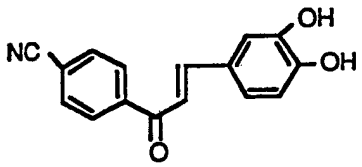

MV-46

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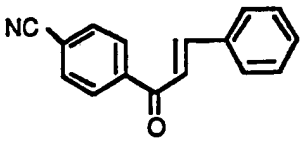


MV-47

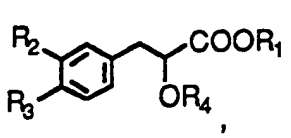
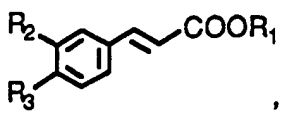
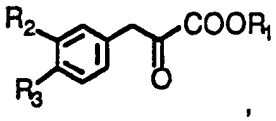
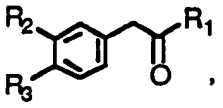
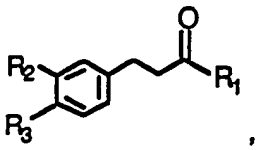
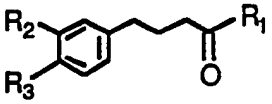
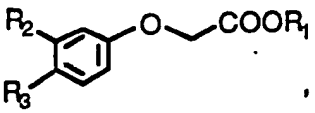
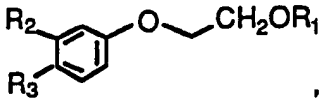
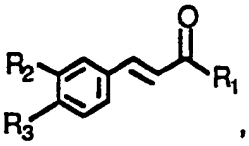
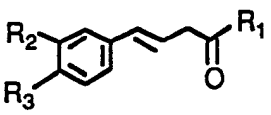
Most preferred compounds of this group for practicing the present invention are 3-(4-hydroxyphenyl)-1-phenyl-2-propen-1-one and 4-(4-hydroxyphenyl)-3-buten-2-one, analogs, chemical derivatives and chemically related compounds and pharmaceutically acceptable salts thereof.

Another embodiment of the present invention includes a method for treating cancer comprising the step of administering a therapeutic dose of MeHPLA, its analogs, chemical derivatives or chemically related compounds. This compound, can be any of the above-described antitumor compounds.

In addition to being used as a treatment for cancer, these antitumor agents are also useful as inhibitors of cell growth and proliferation in those cells which include a Type II nuclear estrogen binding site.

These compounds bind to Type II nuclear estrogen binding sites and regulate cell growth. Specific proliferating cells which are sensitive to the binding of these compounds include estrogen responsive tissues such as uterus, mammary gland, uterine tumors and mammary tumors. The above-described compounds inhibit the proliferative capacity of human breast cancer cells and thus provide an effective therapy for this disease. Benign prostatic hyperplasia is another example of a proliferative tissue disease in which the above-described compounds can successfully be used in the treatment.

Another group of compounds which show tumor anti-proliferative and immunosuppressive action is described by the general formulas: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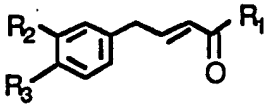
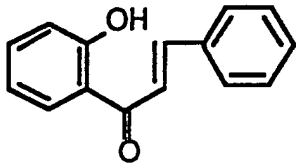
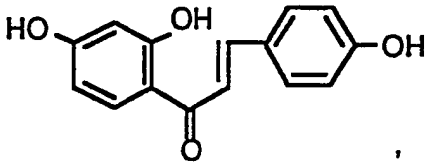


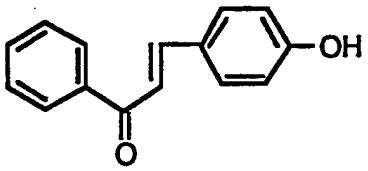
Wherein R_1 is from the group consisting of H, alkyl groups containing 1 to 6 carbons, and substituted or unsubstituted aryl groups; and R_2 and R_3 are selected from the group consisting of H, OH and OCH_3. Preferred compounds of this group which may be used to practice the present invention are:



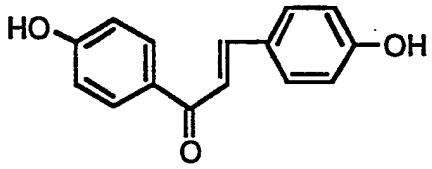
2'-hydroxychalcone



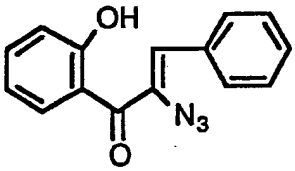
2',4',4-trihydroxychalcone



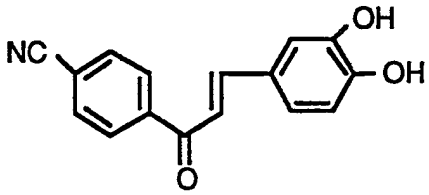
4-hydroxychalcone



4-4'-dihydroxychalcone

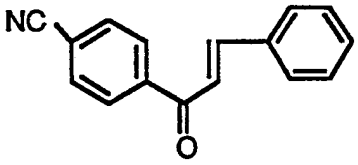


MV-35



MV-46

and



MV-47

Most preferred compounds of this group for practicing the present invention are 2'-hydroxychalcone, 2',4',4,-trihydroxychalcone, 4-hydroxychalcone, 4-4'-dihydroxychalcone, MV-35, MV-46, and MV-47, analogs, chemical derivatives and chemically related compounds and pharmaceutically acceptable salts thereof.

Another embodiment of the present invention includes a method for treating autoimmune diseases comprising the step of administering a therapeutic

dose of MeHPLA, its analogs, including, but not limited to, phenylmethylene ketones, nitroalkenes, aurones, and chalcones, chemical derivatives or chemically related compounds. This compound, can be any of the above-described antitumor or immunosuppressive compounds.

Example 1

[^{3}H]Estradiol Binding Assay

A variety of rat tissues possess an endogenous ligand which blocks [^{3}H]estradiol binding to Nuclear Type II estrogen binding sites; however, this compound does not interfere with [^{3}H]estradiol binding to the estrogen receptor. Uterine tissue from estradiol-implanted rats was dissected from host animals. The uterine nuclei were prepared as previously described in Markaverich, B. M. et al., J. Biol. Chem. 258:11663-11671 (1983), the disclosure of which is herein incorporated by reference. Uterine nuclei were prepared from estrogen-implanted, adult-ovariectomized rats. The washed nuclear pellet was diluted to 10 mg of fresh uterine equivalents/ml. At this concentration the effects of the endogenous inhibitor were minimal, and Nuclear Type II sites bound maximum quantities of [^{3}H]estradiol. Aliquots of these nuclei and various concentrations of the compounds of the present invention were incubated at about 4°C for approximately 60 min in the presence of 40 mM of [^{3}H]estradiol with and without 12 uM diethylstilbestrol. Under these conditions, nuclear Type II sites were quantitatively measured without interference from Type I sites. The nuclear pellets were resuspended in 1 ml of 10 mM Tris-1.5 mM EDTA and centrifuged, ethanol extracted, and counted. The results were expressed as the percentage of [^{3}H]estradiol bound as compared to the buffer control, or as the percentage of inhibition where 100% bound was 0% inhibition and was equivalent to approximately 45,000 cpm. Figure 1 demonstrates representative data showing that concentrations of these compounds above 10nM competed for the [^{3}H]estradiol Nuclear Type II sites. The results represented by Figure 1 represent the mean + standard error of the mean for triplicate determinations in four replicate experiments for each preparation.

Binding assays have shown that the mouse mammary tumor and human breast cancer preparations had high levels of free nuclear Type II sites relative to non-malignant tissues. Normal rat mammary glands contain very high levels of total inhibitor activity relative to mouse mammary tumors. Human breast cancer contains low levels of inhibitor. Thus, the evidence shows that malignant tissues have high levels of free nuclear Type II sites and are deficient in the inhibitor activity. This deficiency in inhibitor activity explains the high levels of free nuclear Type II sites observed in these tumor tissue populations as well as their rapid rate of proliferation, cell growth and DNA synthesis.

It has been previously demonstrated (U.S. Patent Application No. 079,199, incorporated herein by reference) that two endogenous inhibitors (å and ∫) are present in normal tissues such as rat uterus and normal mouse mammary gland. A high correlation between a deficiency in the ∫-peak component and increased unbound Nuclear Type II sites was also demonstrated in rat, mouse and human mammary tumors.

The data presented in Figure 1 demonstrate that compounds such as MV-3, MV-12, and MV-88 bind to nuclear type II binding sites with a very high binding affinity (Kd-1-10 nM), as does MeHPLA. Similar binding inhibition curves were obtained for all of the compounds presented in Table I, below. The Ki's for the binding interaction was determined. The Ki is the concentration of drug which inhibits [^{3}H]estradiol binding to nuclear type II binding sites by 50%. The data for all compounds presented in Table I show a good correlation between binding affinity and cell growth inhibitory activity.

Table I Effects of MV-Compounds on Type II Binding Sites, MCF-7 Human Breast Cancer Cell Proliferation in vitro, and Mouse Mammary Tumor Growth in viv..

Compound	Type II Sites[a] (Ki, mM)	Cell Proliferation[b] (Ki, µg/ml)	Tumor Growth[c]
Phenylmethylene Ketones			
MV-1	34.00	Inactive	Not Tested
MV-3	0.06	1.00	+++
MV-17	0.500	1.60	Not Tested
MV-18	0.800	1.00	Not Tested
Nitroalkenes			
MV-N1	?10.0	1.40	Not Tested
MV-N3	0.220	0.80	Not Tested
Aurones			
MV-19	10.0	8 00	Negative
MV-20	2.800	1.90	+
MV-21	0.08	0.78	+++
Chalcone-Cyclodextrin Complex			
MV-88CD	Not Tested	3.00	+++

[a]Ki is the concentration (mM) of the compound required to inhibit [^{3}H]estradiol binding to Nuclear Type II sites by 50% (see Figure 5). [b]Ki is the concentration (mg/ml) required to inhibit MCF-7 human breast cancer cell proliferation by 50% (see Figure 5). [c]This compound inhibited the growth of well differentiated mouse mammary adenocarcinomas in vivo (see Figures 7-9).

In order to test whether Nuclear Type II binding si:.s were also present in tissues of the immune system, popliteal lymph node nuclei were prepared and analysis of Nuclear Type II binding sites were performed by [^{3}H]estradiol exchange. Popliteal lymph nodes were removed from mature female mice, dissected from extraneous tissue, weighed and homogenized in TE buffer (10 mM Tris; 1.5 mM EDTA, pH 7.4). The homogenate was centrifuged at 800 x g for 20

minutes to obtain the nuclear pellet. The nuclear pellet was washed three times by resuspension and centrifugation in TE buffer (800 x g x 7 minutes), and the final washed nuclear preparation was diluted to a final volume of 30 mg tissue wet weight equivalents/ml (8.6 ml) and assayed for Type II binding sites by [^{3}H] estradiol exchange (Markaverich et al, Endocrinology 109: 62-69 (1981)). Briefly, this consisted of incubating aliquots (250 ml) of the nuclear suspension with [^{3}H] estradiol (4-40 nM) in the absence (total binding) or presence (non-specific binding; NS) at 4°C for 60 minutes. Following incubation, the nuclei were washed 4 times by resuspension and centrifugation in TE buffer, and the final washed nuclear pellets were extracted with 1 ml of ethanol (100%). The ethanol extract was decanted to mini-vials and radioactivity determined by liquid scintillation counting. Specific binding was determined by subtraction of non-specific binding (NS) for the total binding in the system. Results were expressed as picomoles of [^{3}H] estradiol bound per gram wet weight of lymph node tissue and 1 pmole represented 70265 cpm. Figure 2 demonstrates the presence of nuclear type II estrogen binding sites in the popliteal lymph nodes. These type II binding sites in popliteal lymph nodes possessed an equivalent binding affinity (Kd»20nM) to those sites observed in other tissues such as the rat uterus, and were present in equivalent numbers as compared to those measured in other non-estrogenized, non-malignant tissues. Popliteal lymph nodes contain almost exclusively lymphocyte cells and these cells should therefore be inhibited by MeHPLA related analoges and derivatives through binding interactions with nuclear type II sites.

Example 2

In Vitro Inhibition of Cell Proliferation

A. MCF-7 Breast Cancer Cell Proliferation Assay

To assay for tumor growth sensitivity, the MCF-7 human breast cancer cell line in tissue culture was used. One skilled in the art will recognize that this is an excellent model system to assess the effects of hormones and drugs on human cancer cell growth and proliferation. The MCF-7 cells have both Type I and nuclear Type II estrogen receptor sites and respond in a proliferative fashion to

estrogenic hormones. Furthermore, they are inhibited by well-known anti-estrogens such as Tamoxifen. The MCF-7 cells were plated at 5 x 10^5 cells/dish in 30 mm petri dishes and grown in Dulbecco's Modified Eagles Medium containing about 10% charcoal stripped fetal bovine serum for approximately 48 hours. During this interval, the cells attached to the plastic dishes and then underwent exponential growth with a cell-doubling time of approximately 24 hours. The plated cells were allowed to attach for approximately 48 hours and the medium was replaced ("day zero"). The cells were allowed to grow exponentially for about 6 days. At day zero the cells were treated with doses ranging from 0.1-10 ug/ml of the compound of interest, for example, methyl p-hydroxyphenyllactate, in 10 u1 of ethanol. The medium was changed at about 2 and 4 days. The control and test solutions were also re-added when the medium was changed. On day 6 the cells were harvested, counted on a hemocytometer and DNA content per dish was determined by the Burton assay (Burton, K., Biochem. J. 62:315-323, 1956). Results are expressed as cells/dish or DNA content (ug/dish) at 6 days following treatment. The results are shown in Figure 3.

The effects of compounds MV-3, MV-12, and MV-88 on MCF-7 cell proliferation were assessed. MCF-7 cells were plated at 2 x 10^4 cells/well in Dulbecco's Modified Eagles Medium (DMEM) containing 10% fetal calf serum. After 24 hours, the cells were treated with concentrations of MV-3, MV-12 or MV-88 at concentrations ranging from 10^2 to 10^5 ng/ml dissolved in 10 ul ethanol. Controls were treated with an equivalent volume of ethanol. The cell number was determined 48 hrs following treatment. As demonstrated in Figure 3, MV-3, MV-12, and MV-88 also inhibited the proliferation of MCF-7 human breast cancer cells. The proliferation of Y-79 retinoblastoma cells, ME-180 human cervical cancer cells and human melanoma cells was also inhibited (data not shown). Utilizing similar data derived from inhibition curves for all of the compounds shown in Table II, the Ki's for cell inhibition was determined. The Ki for inhibition is defined as the concentration of drug (µg/ml) required to inhibit cell proliferation by 50%.

Table II. INHIBITION OF HUMAN MELANOMA (HSO294 CELLS) AND BREAST CANCER (MCF-7 CELLS) CELL PROLIFERATION BY CHALCONES AND THEIR DERIVATIVES.

Compound	Melanoma Cells [a]	MCF-7Cells [a]
MV-39	0.9	0.9
MV-46	5.0	5.0
MV-47	0.9	6.0
MV-72	3.0	3.0
MV-88	1.5	1.0

[a]Values represent the Ki for inhibition where Ki is the concentration (μg/ml) required to inhibit cell proliferation by 50% (see Figure 5) relative to control (untreated cells).

The effects of inhibition were reversible. It took approximately 24 hours for the cells to recover and about 7 to 24 days after the removal of the ∫-peak inhibitor to regrow to a full monolayer.

B. Melanoma Cells

Analogous experiments were conducted with human melanoma cells, and MV-39, MV-46, MV-47, MV-72 and MV-88 inhibited the proliferation of these cells at relatively low concentrations (Table II). Therefore, chalcones (MV-72 and MV-88), and their cyano (MV-46, MV-47) and azido (MV-35) derivatives also inhibit the proliferation of malignant cells.

It is postulated that tumor cell proliferation is very rapid because the tumor cell metabolizes or inactivates the ∫- peak inhibitor. This is supported by the observation that methyl p-hydroxyphenyllactate is found bound to Type II sites in normal tissues but is not found in malignant tissue. Cell proliferation is regulated by ligand binding to nuclear Type II sites. The number of unbound sites determines the rate of proliferation. Since tumor cells have an increased number of unbound nuclear Type II sites and thus, their growth is not inhibited by the amount of endogenous inhibitor normally present in the cells. Therefore, tumor cell proliferation is dramatically accelerated as compared to the rate of normal cell

proliferation (i.e., cells with fewer or no unbound nuclear Type II sites). These rapidly proliferating cells are brought back into regulation and cell proliferation is decreased by administering a therapeutic dose of the inhibitors described in the present invention. The experimental protocol utilized for assessing the effects on human melanoma cells (HSO294 cells) was identical to that described above for MCF-7 cells.

Example 3

In Vivo Inhibition of Mammary Tumor Growth

To assess drug effects on tumor growth in vivo, a transplantable mouse mammary tumor model system was utilized. This tumor model and its use as an assay for anti-tumor drugs has been previously described by Markaverich et al. Cancer Res. 43:3208 (1983) and is incorporated herein by reference. Briefly, syngeneic female mice bearing transplanted mammary tumors were divided into experimental groups as indicated. When the tumors were approximately 0.5 x 0.5 cm in size (length x width) (day 0), the animals were treated with blank silastic capsules (controls), or silastic capsules containing 25 mg of the test compound. Tumor size (length x width) was monitored at the indicated times following treatment. These implants continuously released 450 ng of the compound daily. Figure 4 demonstrates that MV-3, MV-12 and MV-88 inhibited the growth of these tumors as compared to controls. Similarly, an excellent correlation was observed between binding affinity (Table I) and the anti-tumor activity of MV-19, MV-20 and MV-21 (Figure 19).

Example 4

Utertropic Assay

The rat uterus is exquisitely sensitive to estrogen, and this hormone stimulates uterine cellular hypertrophy, hyperplasia and DNA synthesis within 24 hours following a single injection. Estradiol stimulation of nuclear Type II sites is a prerequisite for these responses. This assay includes injecting immature female rats with saline-ethanol vehicle, estradiol-17β and the test compound of interest.

Control rats were injected only with saline-ethanol vehicle and estradiol-17∫. The rats were sacrificed 24 hours later and the uterine wet and dry weights were determined. The wet and dry weight measurements are well defined biochemical end points of estrogen action and are a direct index of changes in cell proliferation and DNA synthesis. The results of these experiments with various compounds are shown in Table III.

Table III. MeHPLA Analogue and Related Compound Effects on Uterine Growth and Nuclear Type II Site Binding Inhibition

COMPOUND	GROWTH INHIBITION [a]	TYPE II INHIBITION [b]
methyl 3-(4-hydroxyphenyl) 2-hydroxypropionate	90.0	0.8
3-(4-hydroxyphenyl)-2-hydroxypropionic acid	0.0	80.0
1-(4-hydroxyphenyl)-3-butanone	96.0	2.0
methyl 3-(3,4-dihydroxyphenyl)-2-propenoate	70.0	1.0
methyl 3-(4-hydroxy-3-methoxyphenyl)-2-propenoate	56.0	6.0
methyl (4-hydroxyphenoxy)acetate	70.0	0.8

[a] Determined by the ability of the compound (10μg) to block estradiol stimulation in the rat uterotropic assay.

[b] The concentration (nM x 10^{-2}) of the compound to inhibit nuclear Type II binding sites by 50%.

Additional in vivo measurements using the uterotropic assay show the utility of these compounds for inhibiting cell proliferation. Low doses of methyl p-hydroxyphenyllactate, but not hydroxyphenyllactic acid, block estradiol stimulation of uterine growth in the immature rat (Figure 14 and Table III). However, higher doses of hydroxyphenyllactic acid showed some partial antagonism. This is not surprising since it is known that hydroxyphenyllactic acid

binds to nuclear Type II sites with a 20-fold lower affinity than methyl p-hydroxyphenyllactate.

Previous results demonstrated that the bound/unbound ratio of nuclear Type II sites is important in the regulation of cell growth and that the primary bound inhibitor in normal cells is methyl p-hydroxyphenyllactate (Markaverich et al. 1988).

Since the data demonstrated that tumor cells have the ability to inactivate methyl p-hydroxyphenyllactate, the analogues and chemically related compounds described in this invention were synthesized to avoid this inactivation. Thus, administration of compounds with various side-chains and various substituents on the aromatic ring resulted in inhibition of uterine growth.

One compound 1-(4-hydroxyphenyl)-3-butanone (p-hydroxyphenylbutanone) which includes a C-terminal methyl group is not subject to the esterase cleavage since the methyl group is attached by a C-C bond. This compound is more stable and thus a better inhibitor in culture and in vivo. Furthermore, experiments with 1-(4-hydroxyphenyl)-3-butanone demonstrated that it binds to the nuclear Type II sites with a high affinity and blocks estradiol stimulation of uterine growth when injected into immature rats. Thus, 1-(4-hydroxyphenyl)-3-butanone is an effective inhibitor of tumor growth and regulator of cell proliferation.

Many of the compounds of the present invention, particularly phenylmethylene ketones, nitroalkenes, aurones, and chalcones, have very low solubility in aqueous solutions such as those commonly used as injection vehicles. This insolubility causes major problems with drug delivery and potential use clinically. To improve drug solubility and delivery, the compounds may be encapsulated to improve their delivery. Among the types of drug delivery systems useful in the present invention are incorporation into liposomes and encapsulation in cyclodextrins.

Example 5

Enhancement of Delivery of Compounds of Present Invention

Cyclodextrins solubilize hydrophobic compounds and improve gastrointestinal absorption (Szejtli, (1982) In: "Cyclodextrins and Their Inclusion Complexes", Akadmiai Kiado, Budapest, Hungary). Bioflavonoids such as quercetin (closely related to MV-88) have recently been administered by this method (Yan, et al (1988) Zhongcaoyoa, 19:492). The lack of cytotoxicity of 2-hydroxypropyl ∫-cyclodextrin and poly ∫-cyclodextrin (a soluble form of ?-cyclodextrin) has been demonstrated in animals (Pitha and Pitha (1985) J. Pharm. Sci. 19:492) and to a limited extent in humans (Pitha, J. (1984) Third Internat. Sympos. on Clathrate Compounds, Tokyo, p69). These compounds increased the efficacy and sustained delivery of the MV-88 related enones and chalcones. Cyclodextrin was condensed with propylene oxide in aqueous alkali to give 2-hydroxycyclodextrin as described in Pitha and Pitha (1985). The drug was added in excess (2 fold excess, i.e., 200 mg drug to 100 mg 2-hydroxycyclodextrin) to a solution of 2-hydroxycyclodextrin dissolved in water or saline. The drug was added to the aqueous cyclodextrin solution in methanol. The suspension was then stirred at room temperature, and the excess non-solubilized drug was removed by centrifugation and ultrafiltration as described in (Pitha and Pitha (1985) J. Pharm. Sci. 19:492). Quantitation of drug encapsulated in the cyclodextrin preparations was by high performance liquid chromatography (HPLC). Briefly, this consisted of weighing out the dried cyclodextrin-drug mixture and dissolving known amounts (1 mg/ml) of the preparation in methanol. This procedure extracted the drug from the cyclodextrin such that MV-88 could be quantitated by HPLC. For HPLC analysis, known concentrations of MV-88 standards (1-20ug), or aliquots (5-50ul) of the cyclodextrin-MV-88 preparation were injected onto a Waters uBondapak C18 column and eluted with water: methanol (30:70) at a flow rate of 1 ml per minute. The area of the sample peaks detected at 268 nM versus the known MV-88 standards allowed quantitation of the concentration of the MV-88 encapsulated in the cyclodextrin. Peak areas of the MV-88 standards and sample preparation injections were determined by measuring the peak height (in cm) and multiplying by the peak width (in cm) at 1/2 height (area=cm^2). In this manner a standard curve was constructed (MV-88 concentration versus OD 268 reading)

and the concentration of MV-88 in the sample (OD 268 reading) was determined from the slope of the standard curve. Typical values were approximately 1 mg MV-88 per 12 mg cyclodextrin MV-88 complex.

To test the effectiveness of the compounds of the present invention to inhibit tumor growth when encapsulated in cyclodextrin, MV-88 encapsulated in cyclodextrin was dissolved in saline vehicle and administered by subcutaneous injection to mammary tumor bearing mice. MV-88 is insoluble under aqueous conditions. However, MV-88 cyclodextrin (MV-88 CD; Table II) inhibited mouse mammary tumor growth in a dose-dependent fashion (Figure 6). When administered by injection in other vehicles such as dimethylsulfoxide saline, MV-88 was not as effective at inhibiting the growth of this tumor as when administered after cyclodextrin encapsulation. However, MV- 88, when administered in a continuous fashion by silastic implant (Figure 4), significantly inhibited tumor growth.

Mice bearing transplantable mammary tumors were implanted with silastic capsules containing 25 mg of the test compound. Controls were implanted with blank capsules and tumor size (length x width) was determined at the 0, 3, 6 and 14 days following implantation. The capsules releases approximately 450 ng of test compound per day (about 10-15 mg/Kg of body weight). No significant effects on the body weights of the treated animals relative to controls were observed throughout the experimental period. Figure 4 demonstrates that treatment of mouse mammary tumors with MV-3, MV-12, and MV-88 in silastic implants caused a complete inhibition of the growth of the mammary tumor in vivo. However, treatment of mice bearing transplantable mammary tumor with silastic capsules containing MV-19 and MV-20 caused no significant growth inhibition relative to the controls because MV-19 and MV-20 are not released efficiently from the silastic capsules. Therefore, these compounds were administered by cyclodextrin encapsulation procedures which enhanced their solubility in aqueous injection vehicles. These structure activity studies demonstrated that there was a precise structure/activity relationship between binding affinity and tumor growth inhibition observed with MV-19, MV-20 and MV-21. MV-19 and MV-20 did not bind to nuclear type II sites with high affinity, and did not inhibit mammary tumor growth. However, MV-21 was found

to bind with a relatively high affinity, and treatment with MV-21 caused a significant inhibition of the tumor growth. (Figure 5)]. It is likely that alteration of the treatment regime and drug dosage will cause complete inhibition of tumor growth.

Example 6

Immunosuppressive Activity

Many anti-cancer drugs possess immunosuppressive activity (Seldin and Steinberg (1988) In: "Inflammation Basic Principles and Clinical Correlates", (Galin, J.I., Goldstein, M., Snyderman, R., eds.) Raven Press, Ltd., New York). Immunosuppressive drugs have proven to be therapeutically effective in treating a variety of autoimmune diseases. In the United States, 10-15% of all blindness is caused directly or indirectly by inflammation of the uveal tract or uveitis. Unless therapeutic intervention in uveoretinitis is initiated, irreversible damage can occur resulting in reduced retinal function and/or blindness. The administration of corticosteroids is currently the most effective treatment for uveoretinitis. However, use of corticosteroids is often associated with side effects such as elevated intraocular pressure and cataract formation. Immunosuppressive drugs used heretofore including cyclosporin-A (csA) which blocks the proliferation of T-lymphocytes by interference with the Interleukin-2 receptor expression produce adverse side effects such as hepatotoxicity and nephrotoxicity.

The compounds of the present invention such as MV-3 were tested for immunosuppressive activity using the established rat model of experimental autoimmune uveoretinitis (Gery et al. (1986) Invest. Opthalmol. Vis. Sci. 103: 1559). Female Lewis rats weighing 100-200 grams were immunized with Interphotoreceptor Retinoid Binding Protein (IRBP) peptide corresponding to amino acid positions 523-538 of the bovine and human molecules. This sequence designated #896 has the amino acid sequence LTSHRTATAAEEFAFL. the rats were immunized by a single foot pad injection of 50 micrograms of IRBP #896 emulsified in complete Freund's adjuvant containing 2.0 mg/ml M. tuberculosis H37Ra (Difco). The rats simultaneously received $10x10^9$ heat-killed B. pertussis organisms by intraperitoneal injection. 100% of the control, untreated animals

developed severe, bilateral anterior uveitis and posterior uveoretinitis by day 9 or day 10 following immunization. Experimental animals were given intraperitoneal injections of 10 mg of MV-3 on Days 0, 3 and 7.

The MV-3 potent immunosuppressive activity is demonstrated by experiments illustrated in Figure 7. Figures 7a and 7b demonstrate that 9 days after immunization of Lewis rats with interphotoreceptor retinoid binding protein (IRBP) peptide #896 there is a 100% induction of severe, bilateral, panuveitis. In contrast, the disease in MV-3 treated rats (Figure 7c) was totally blocked. There was only a mild uveitic response in some of the MV-3 treated rats at Day 15 (Figure 7 c). These results indicate that there is suppression of the autoimmune response at its usual point of onset with a lag period in which only mild disease develops approximately 6 days later, apparently occurring after active levels of the MV-3 have diminished. In addition, the typical inflammatory response at the foot pad immunization site in MV-3 treated rats was also inhibited. These results suggest that MV-3 and related compounds also have anti-inflammatory properties. MV-3 as well as other compounds of the present invention provide a useful method for treating and/or preventing other autoimmune diseases, including, but not limited to, rheumatoid arthritis, multiple sclerosis, myasthenia gravis, diabetes mellitis, thyroiditis, systemic lupus erythematosus, Sjorgen's syndrome, autoimmune skin diseases, and others. In addition, MV-3 and related compounds provide a useful treatment for graft vs host disease and prevention of transplant rejections.

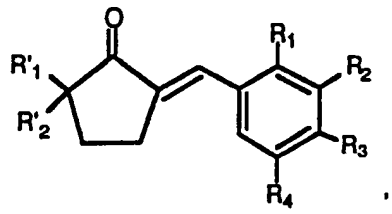
One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The compounds, methods, procedures and techniques described herein are presently representative of the preferred embodiments, are intended to be exemplary, and are not intended as limitations on the scope of the present invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the appended claims.

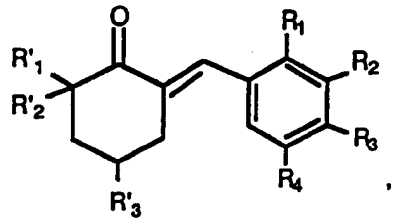
One skilled in the art will readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages

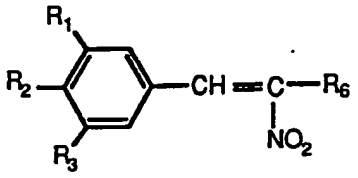
mentioned, as well as those inherent therein. The compounds, methods, procedures and techniques described herein are presently representative of the preferred embodiments, are intended to be exemplary, and are not intended as limitations on the scope of the present invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention and are defined by the scope of the appended claims.

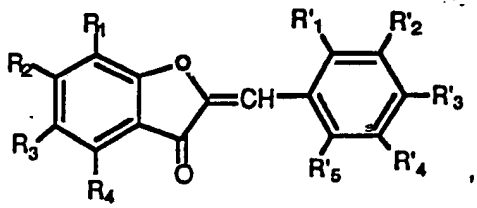
Claims:

1. A method of treating cancer, comprising the step of administering a therapeutic dose of a compound selected from the group consisting of:

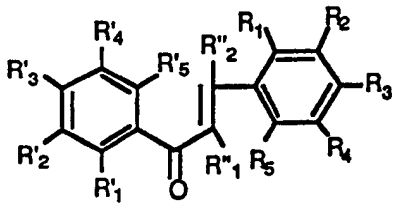






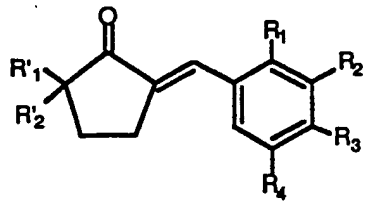


and

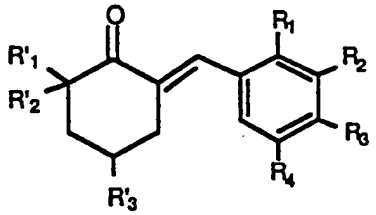


Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido and aziridine derivatives, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

2. The method of Claim 1, wherein said compound is selected from the group consisting of the formulae:

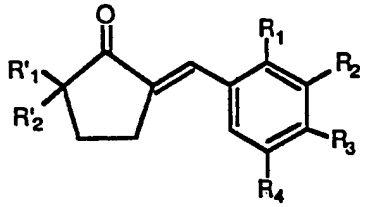


and



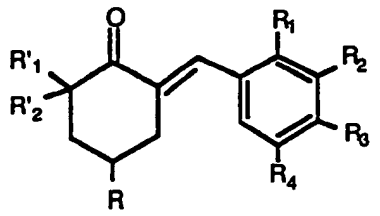
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

3. The method of Claim 1, wherein said compound is :



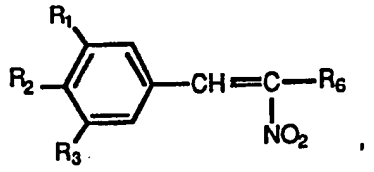
Wherein, R1 and R4 are selected from the group consisting of H and OH; R2, and R3 are selected from the group consisting of H, OH, OCH3, amino, alkylamino and alkyl groups containing 1 to 6 carbons; and R'1 and R'2 are selected from the group consisting of H, CH3, OH, OCH3, 3,4 dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups.

4. The method of Claim 1, wherein said compound is



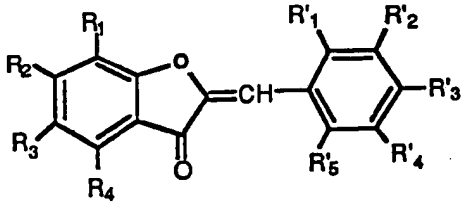
Wherein, R1 and R4 are selected from the group consisting of H and OH; R2, R3 and R'3 are selected from the group consisting of H, OH, OCH3, amino, alkylamino and alkyl groups containing 1 to 6 carbons; and R'1 and R'2 are selected from the group consisting of H, CH3, OH, OCH3, 3,4 dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups.

5. The method of Claim 1, wherein said compound is:



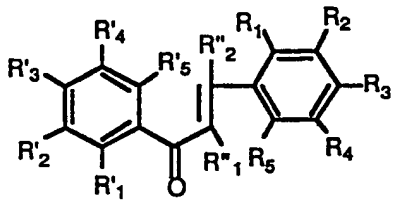
Wherein R_1, R_2 and R_3 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy and halogens, and R_6 is H, or alkyl group of 1 to 6 carbon atoms.

6. The method of Claim 1, wherein said compound is:



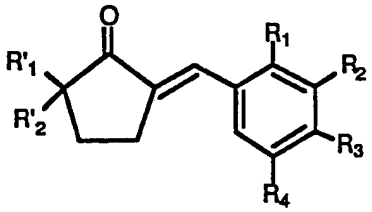
Wherein R_1-R_4 and R'_1-R'_5 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms.

7. The method of Claim 1, wherein said compound is:

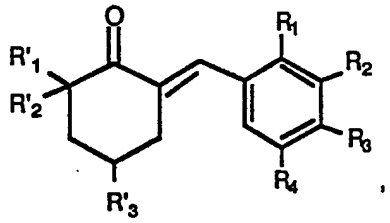


Wherein R_1-R_5, R'_1-R'_5, R''_1 and R''_2 are H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

8. A method for inhibiting the growth of proliferating cells which include a Type II nuclear estrogen binding site comprising the step of administering a biologically inhibiting dose of a compound selected from the group consisting of:



,



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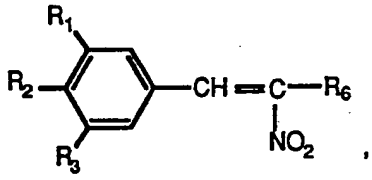

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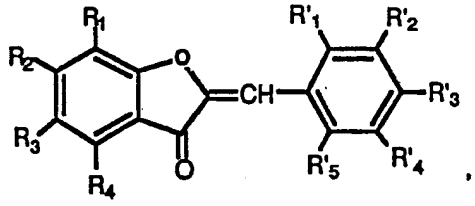

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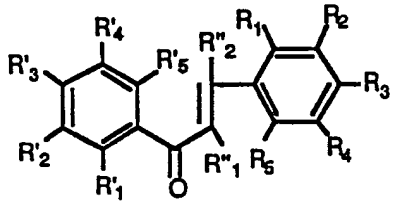
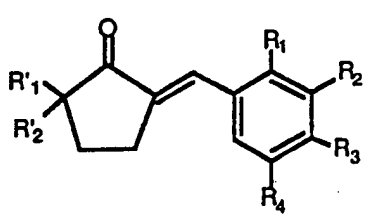
and



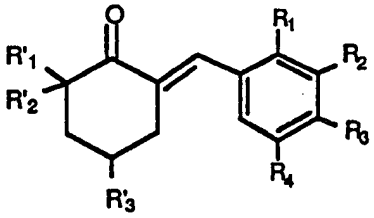
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino,

alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

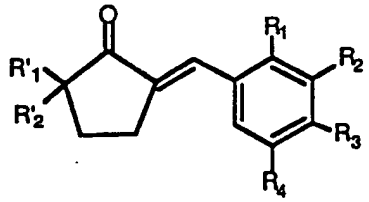
9. The method of Claim 8, wherein said compound is selected from the group consisting of the formulae:



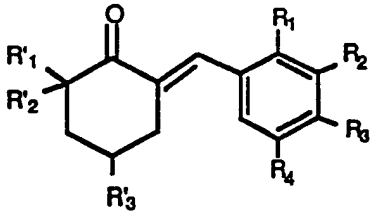
and



10. The method of Claim 9, wherein said compound is selected from the group consisting of the formulae:

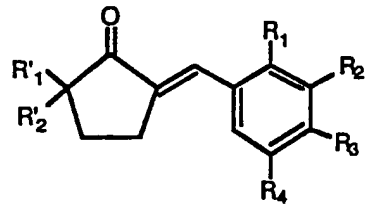


and



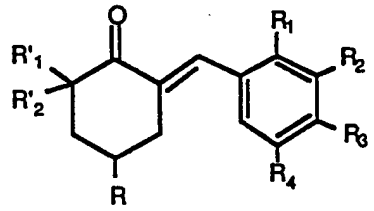
Wherein, R1 and R4 are selected from the group consisting of H and OH; R2, R3 and R'3 are selected from the group consisting of H, OH, OCH3, amino, alkylamino and alkyl groups containing 1 to 6 carbons; and R'1 and R'2 are selected from the group consisting of H, CH3, OH, OCH3, 3,4 dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups.

11. The method of Claim 9, wherein said compound is :



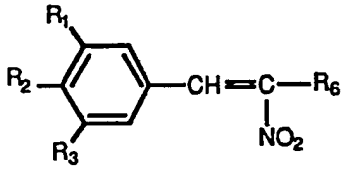
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, and R_3 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons; and R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4 dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups.

12. The method of Claim 8, wherein said compound is



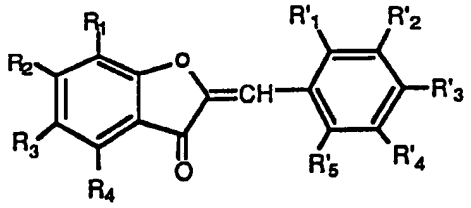
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring-subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

13. The method of Claim 8, wherein said compound is:



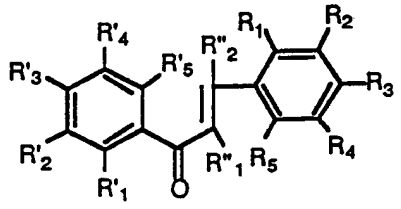
Wherein R_1, R_2 and R_3 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy and halogens, and R_6 is H, or alkyl group of 1 to 6 carbon atoms.

14. The method of Claim 8, wherein said compound is:



Wherein R_1-R_4 and R'_1-R'_5 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms.

15. The method of Claim 8, wherein said compound is:

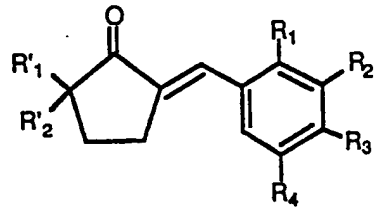


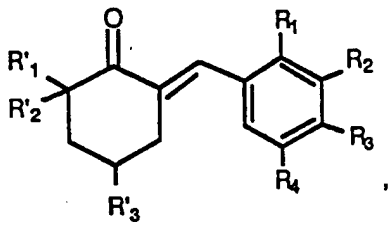
Wherein R_1-R_5 and R'_1-R'_5, R''_1 and R''_2 are H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

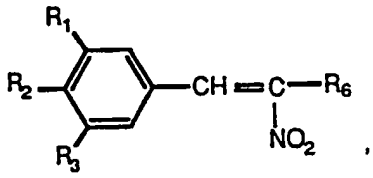
16. The method of Claim 8, wherein said proliferating cells are estrogen responsive tissue selected from the group consisting of uterus, mammary gland, uterine tumors and mammary tumors.

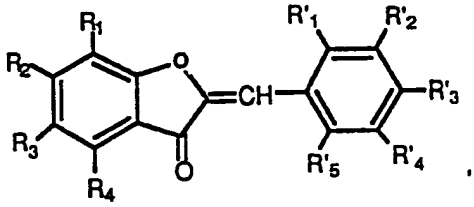
17. The method of Claim 16, wherein said estrogen responsive tissue is human breast cancer cells.

18. A method for treating benign prostatic hyperplasia, comprising the step of administering a therapeutic dose of a compound selected from the group consisting of:

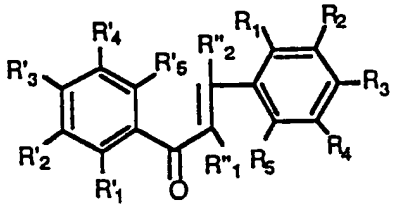






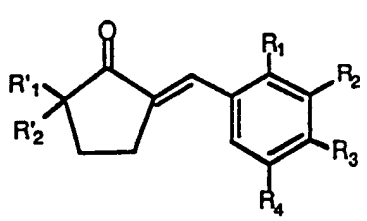


and

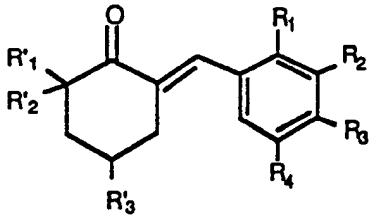


Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

19. The method of Claim 18, wherein said compound is selected from the group consisting of the formulae:

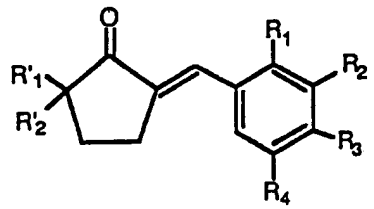


and



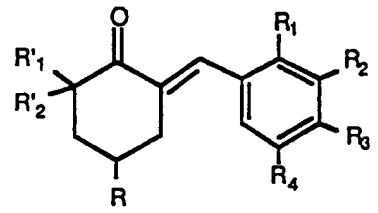
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

20. The method of Claim 18, wherein said compound is :



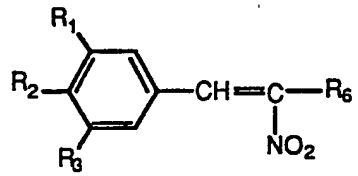
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, and R_3 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons; and R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4 dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups.

21. The method of Claim 18, wherein said compound is



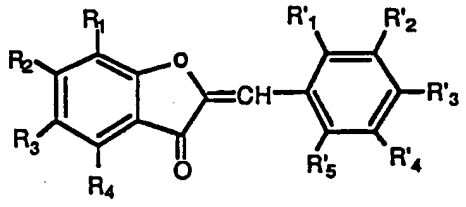
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R'_3 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons; and R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4 dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups.

22. The method of Claim 18, wherein said compound is:



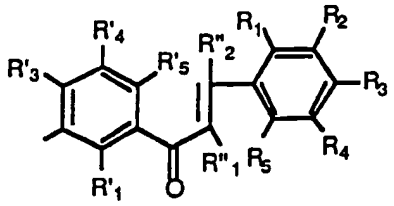
Wherein R_1, R_2 and R_3 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy and halogens, and R_6 is H, or alkyl group of 1 to 6 carbon atoms.

23. The method of Claim 18, wherein said compound is:



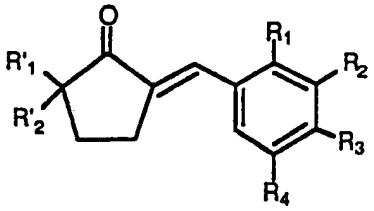
Wherein R_1-R_4 and R'_1-R'_5 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms.

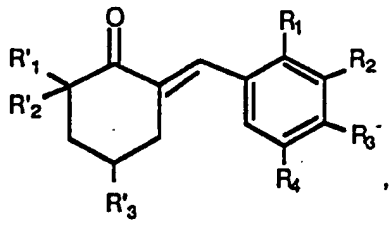
24. The method of Claim 18, wherein said compound is:

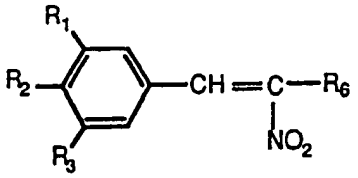


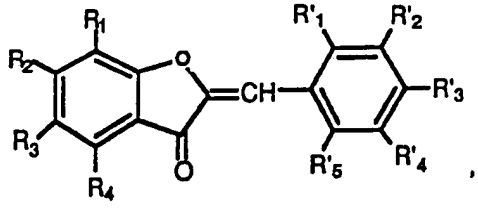
Wherein R_1-R_5, R'_1-R'_5, R''_1 and R''_2 are H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

25. A method of administration of a therapeutic dose of a compound selected from the group consisting of:

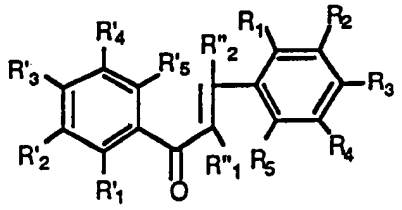






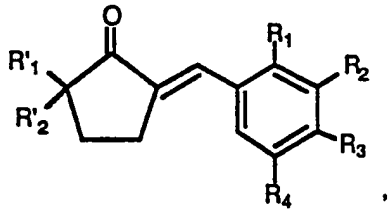


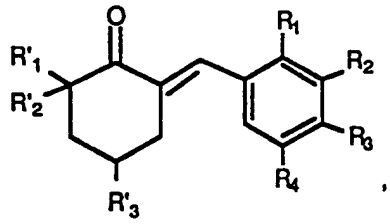
and

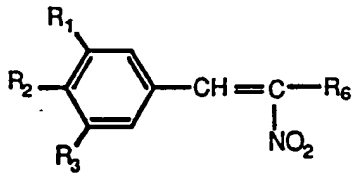


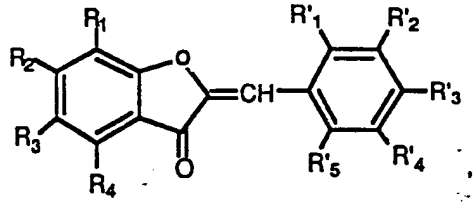
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

26. A method for inhibiting the growth of proliferating cells comprising administration of a biologically inhibiting dose of a compound selected from the group consisting of:

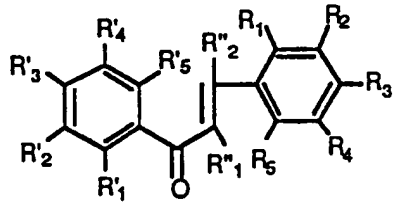






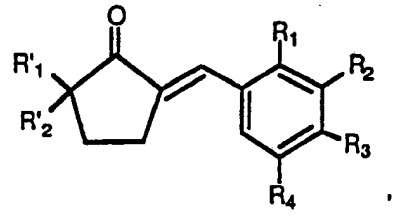


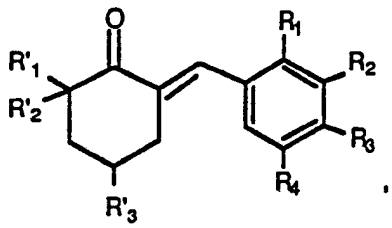
and

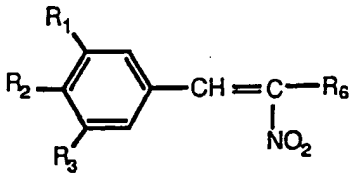


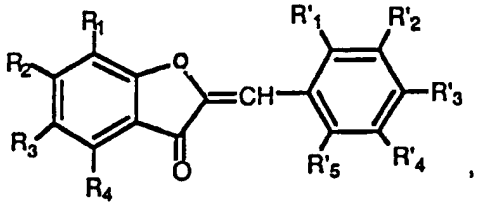
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5 is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

27. A method of treating autoimmune disease, comprising the administration of a therapeutic dose of a compound selected from the group consisting of:

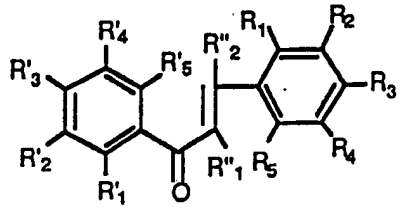








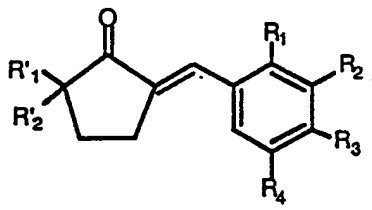
and



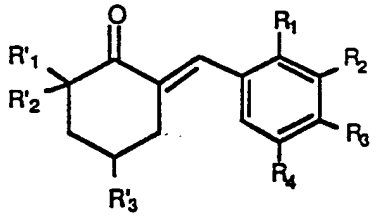
Wherein, R_1 and R_4 are selected from the group consisting of H and OH; R_2, R_3 and R_6 are selected from the group consisting of H, OH, OCH_3, amino, alkylamino and alkyl groups containing 1 to 6 carbons, acyloxy, and halogens; R_5

is selected from the group consisting of H, OH, OCH_3, acyloxy and halogens; R'_1 and R'_2 are selected from the group consisting of H, CH_3, OH, OCH_3, 3,4-dihydroxyphenylmethylene, p-hydroxyphenylmethylene and other substituted phenyl groups preferably with acyloxy or halogen substituents; R'_3, R'_4, R'_5, R''_1 and R''_2 are selected from the group consisting of H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

28. The method of Claim 27, wherein said compound is selected from the group consisting of the formulae:

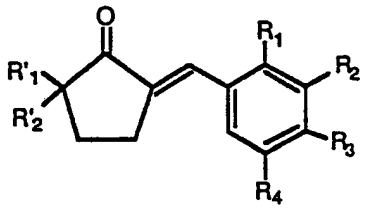


and



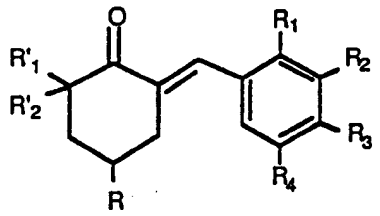
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

29. The method of Claim 27, wherein said compound is :



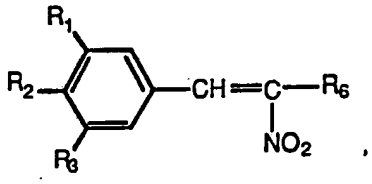
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

30. The method of Claim 27, wherein said compound is



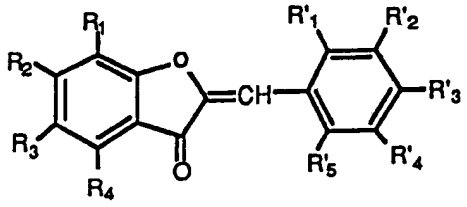
Wherein R_1, and R_4 are H; OH, acyloxy or halogens, R_2 and R_3 are OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms or halogens, R'_1, and R'_2 are H, CH_3, or phenylmethylene having p-hydroxy, 3,4-dihydroxy, acyloxy and halogen ring subtituents and R'_3 is H, OH, CH_3 ,OCH_3 and alkyl groups from 1-6 carbon atoms.

31. The method of Claim 27, wherein said compound is:



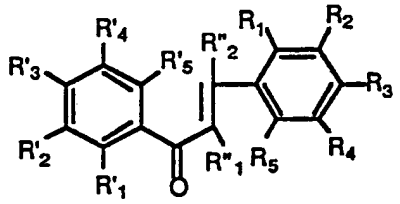
Wherein R_1, R_2 and R_3 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy and halogens, and R_6 is H, or alkyl group of 1 to 6 carbon atoms.

32. The method of Claim 27, wherein said compound is:



Wherein R_1-R_4 and R'_1-R'_5 are H, OH, OCH_3, amino, alkyl or alkylamino groups of 1 to 6 carbon atoms.

33. The method of Claim 27, wherein said compound is selected from the group consisting of:



Wherein R_1-R_5 and R'_1-R'_5, $R"_1$ and $R"_2$ are H, OH, OCH_3, amino, cyano, alkyl or alkylamino groups of 1 to 6 carbon atoms, acyloxy, halogens, å-azido, aziridine, acyloxy and halogen substituted derivatives.

34. The method of any of claims 27-33 wherein said disease is selected from the group consisting of rheumatoid arthritis, multiple sclerosis, myasinia gravis, diabetes mellitus, thyroiditis, uveoretinitis, systemic lupus erythemytosus, Sjorgins Syndrone, autoimmune skin diseases and graft versus host disease and organ and tissue transplant rejections.

35. A method of treating cancer, comprising the step of administering a therapeutic dose of a compound selected from the group consisting of: 2-(hydroxybenzylidene)-5-methyl-cyclopentanone (MV-1), 2,6-bis(4-hydroxybenzylidene)-4-methyl-cyclohexanone (MV-17), 2,6-bis(3,4-dihydroxybenzylidene)-4-methyl cyclohexanone (MV-18), 4-hydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N1), 3,4-dihydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N3), aurone (MV-19), 4'-hydroxyaurone (MV-20), 3',4-dihydroxyaurone (MV-21), 2'-hydroxychalcone (MV-72), 2',4',4-trihydroxychalcone (RV-40), 4-hydroxychalcone (RV-73), 4,4'-hydroxychalcone (MV-88), å-azido-2'-hydroxychalcone (MV-35), 3,4-dihydroxy-4'-cyanochalcone (MV-46) and 4'-cyanochalcone (MV-47).

36. A method for inhibiting the growth of proliferating cells which include a Type II nuclear estrogen binding site comprising the step of administering a biologically inhibiting dose of a compound selected from the group consisting of: 2-(hydroxybenzylidene)-5-methyl-cyclopentanone (MV-1), 2,6-bis(4-hydroxybenzylidene)-4-methyl-cyclohexanone (MV-17), 2,6-bis(3,4-dihydroxybenzylidene)-4-methyl cyclohexanone (MV-18), 4-hydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N1), 3,4-dihydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N3), aurone (MV-19), 4'-hydroxyaurone (MV-20), 3',4-dihydroxyaurone (MV-21), 2'-hydroxychalcone (MV-72), 2',4',4-trihydroxychalcone (RV-40), 4-hydroxychalcone (RV-73), 4,4'-hydroxychalcone (MV-88), å-azido-2'-hydroxychalcone (MV-35), 3,4-dihydroxy-4'-cyanochalcone (MV-46) and 4'-cyanochalcone (MV-47).

37. A method for treating benign prostatic hyperplasia, comprising the step of administering a therapeutic dose of a compound selected from the group consisting of: 2-(hydroxybenzylidene)-5-methyl-cyclopentanone (MV-1), 2,6-bis(4-hydroxybenzylidene)-4-methyl-cyclohexanone (MV-17), 2,6-bis(3,4-dihydroxybenzylidene)-4-methyl cyclohexanone (MV-18), 4-hydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N1), 3,4-dihydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N3), aurone (MV-19), 4'-hydroxyaurone (MV-20), 3',4-dihydroxyaurone (MV-21), 2'-hydroxychalcone (MV-72), 2',4',4-trihydroxychalcone (RV-40), 4-hydroxychalcone (RV-73), 4,4'-hydroxychalcone (MV-88), å-azido-2'-hydroxychalcone (MV-35), 3,4-dihydroxy-4'-cyanochalcone (MV-46) and 4'-cyanochalcone (MV-47).

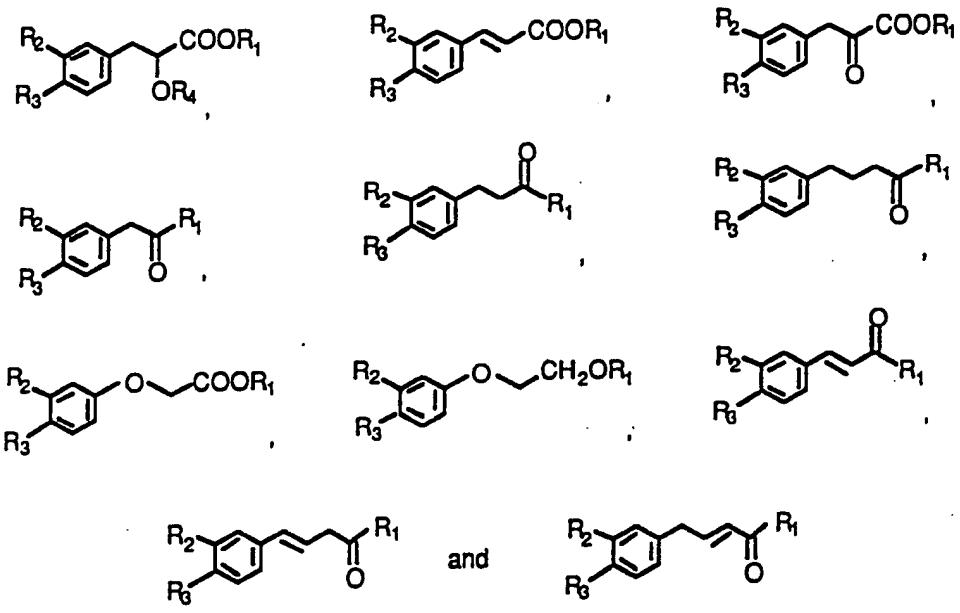
38. A method for inhibiting the growth of proliferating cells comprising administration of a biologically inhibiting dose of a compound selected from the group consisting of: 2-(hydroxybenzylidene)-5-methyl-cyclopentanone (MV-1), 2,6-bis(4-hydroxybenzylidene)-4-methyl-cyclohexanone (MV-17), 2,6-bis(3,4-dihydroxybenzylidene)-4-methyl cyclohexanone (MV-18), 4-hydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N1), 3,4-dihydroxy-ʃ-methyl-ʃ-nitrostyrene (MV-N3), aurone (MV-19), 4'-hydroxyaurone (MV-20), 3',4-dihydroxyaurone (MV-21), 2'-hydroxychalcone (MV-72), 2',4',4-trihydroxychalcone (RV-40), 4-hydroxychalcone (RV-73), 4,4'-hydroxychalcone (MV-88), å-azido-2'-

hydroxychalcone (MV-35), 3,4-dihydroxy-4'-cyanochalcone (MV-46) and 4'-cyanochalcone (MV-47).

39. A method of treating autoimmune disease, comprising the administration of a therapeutic dose of a compound selected from the group consisting of: 2-(hydroxybenzylidene)-5-methyl-cyclopentanone (MV-1), 2,6-bis(4-hydroxybenzylidene)-4-methyl-cyclohexanone (MV-17), 2,6-bis(3,4-dihydroxybenzylidene)-4-methyl cyclohexanone (MV-18), 4-hydroxy-∫-methyl-∫-nitrostyrene (MV-N1), 3,4-dihydroxy-∫-methyl-∫-nitrostyrene (MV-N3), aurone (MV-19), 4'-hydroxyaurone (MV-20), 3',4-dihydroxyaurone (MV-21), 2'-hydroxychalcone (MV-72), 2',4',4-trihydroxychalcone (RV-40), 4-hydroxychalcone (RV-73), 4,4'-hydroxychalcone (MV-88), å-azido-2'-hydroxychalcone (MV-35), 3,4-dihydroxy-4'-cyanochalcone (MV-46) and 4'-cyanochalcone (MV-47).

40. A method for treating autoimmune disease comprising the step of administering a biologically inhibiting dose of a compound selected from the group consisting of methyl p-hydroxyphenyllactate, analogues of methyl p-hydroxyphenyllactate, chemical derivatives of methyl p-hydroxyphenyllactate, and chemically related compounds to the proliferating cells.

41. The method of claim 40, wherein said compound is selected from the group consisting of the formulae:



and

wherein, R_1 is selected from the group consisting of H, alkyl groups containing 1 to 6 carbons and aryl groups; R_2 and R_3 are selected from the group consisting of H, OH, OCH_3; and R_4 selected from the group consisting of H and a alkyl group of 1 to 6 carbons.

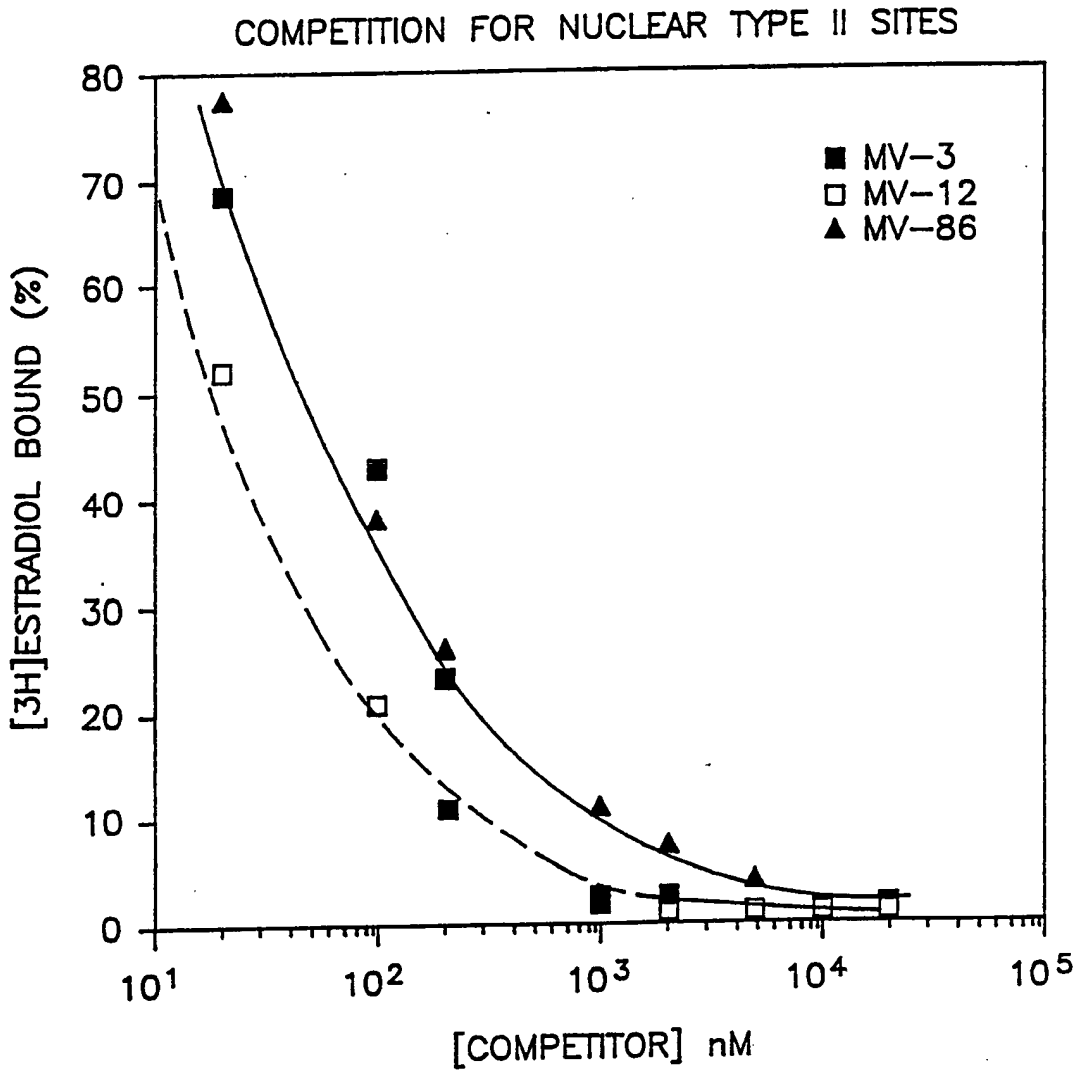

COMPETITION FOR NUCLEAR TYPE II SITES
[3H]ESTRADIOL BOUND (%)
80
70
60
50
40
30
20
10
0
MV–3
MV–12
MV–86
10^1
10^2
10^3
10^4
10^5
[COMPETITOR] nM

FIG. 1

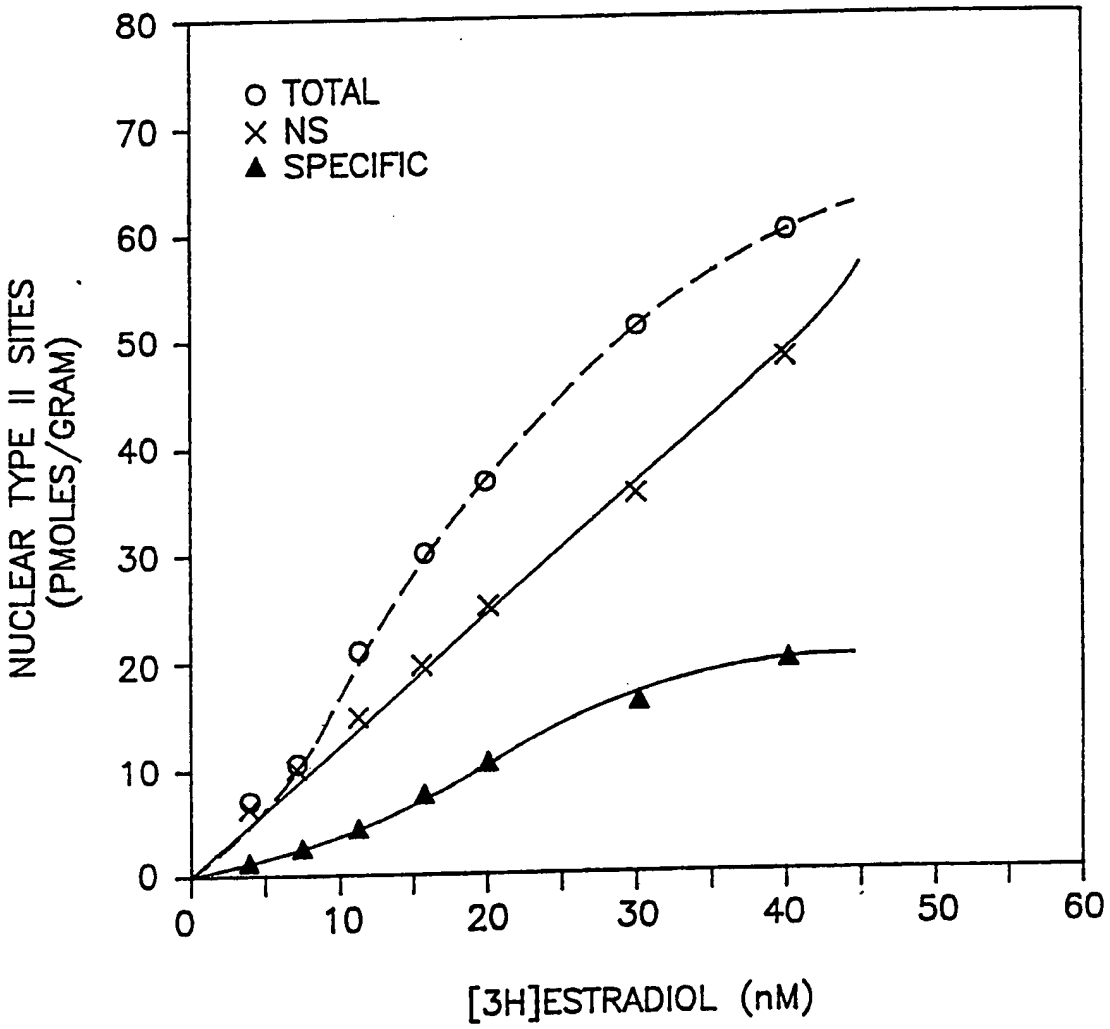

80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
o TOTAL
× NS
▲ SPECIFIC
NUCLEAR TYPE II SITES
(PMOLES/GRAM)
[3H]ESTRADIOL (nM)

FIG. 2

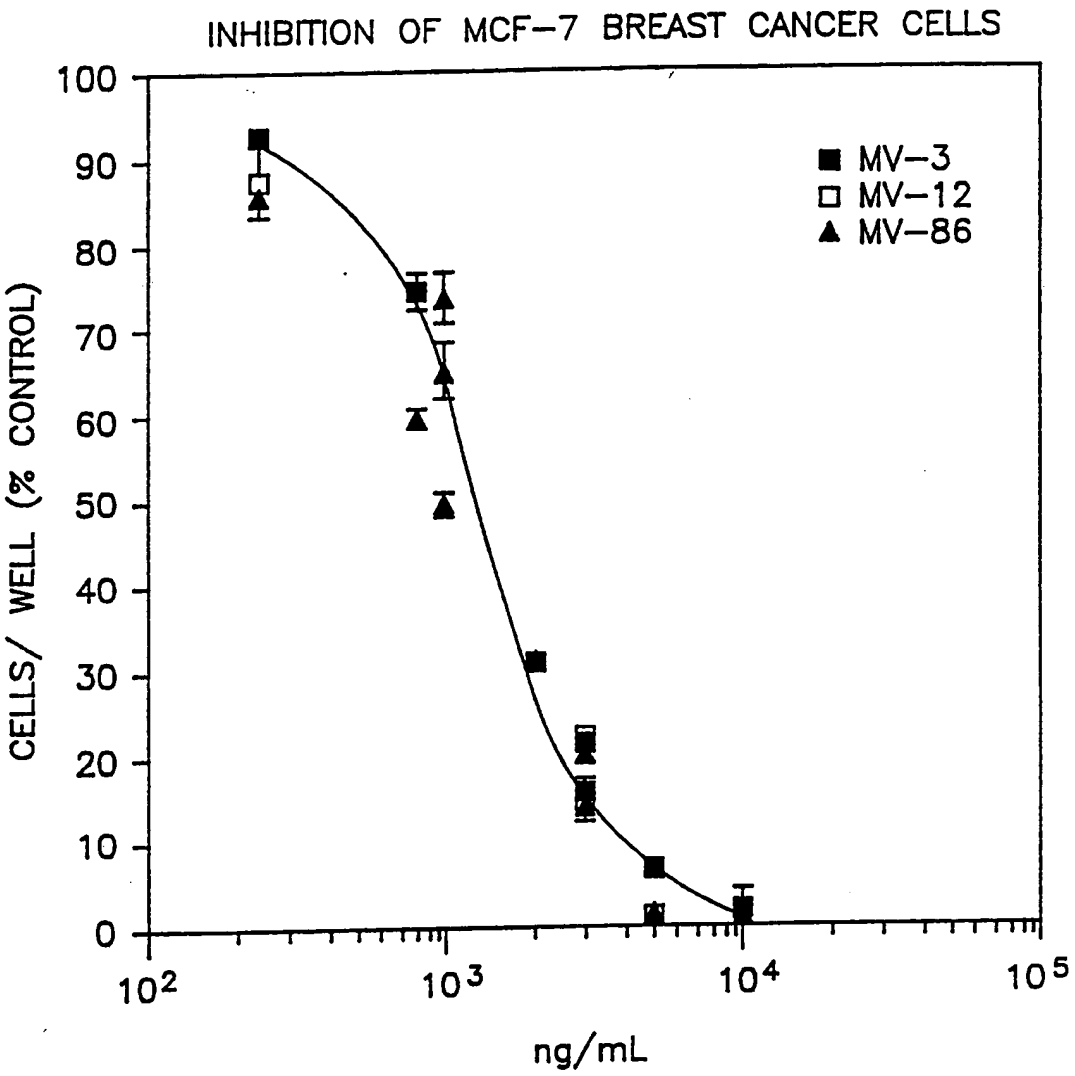

INHIBITION OF MCF-7 BREAST CANCER CELLS
CELLS/ WELL (% CONTROL)
100
90
80
70
60
50
40
30
20
10
0
10^2
10^3
10^4
10^5
ng/mL
MV-3
MV-12
MV-86

FIG. 3

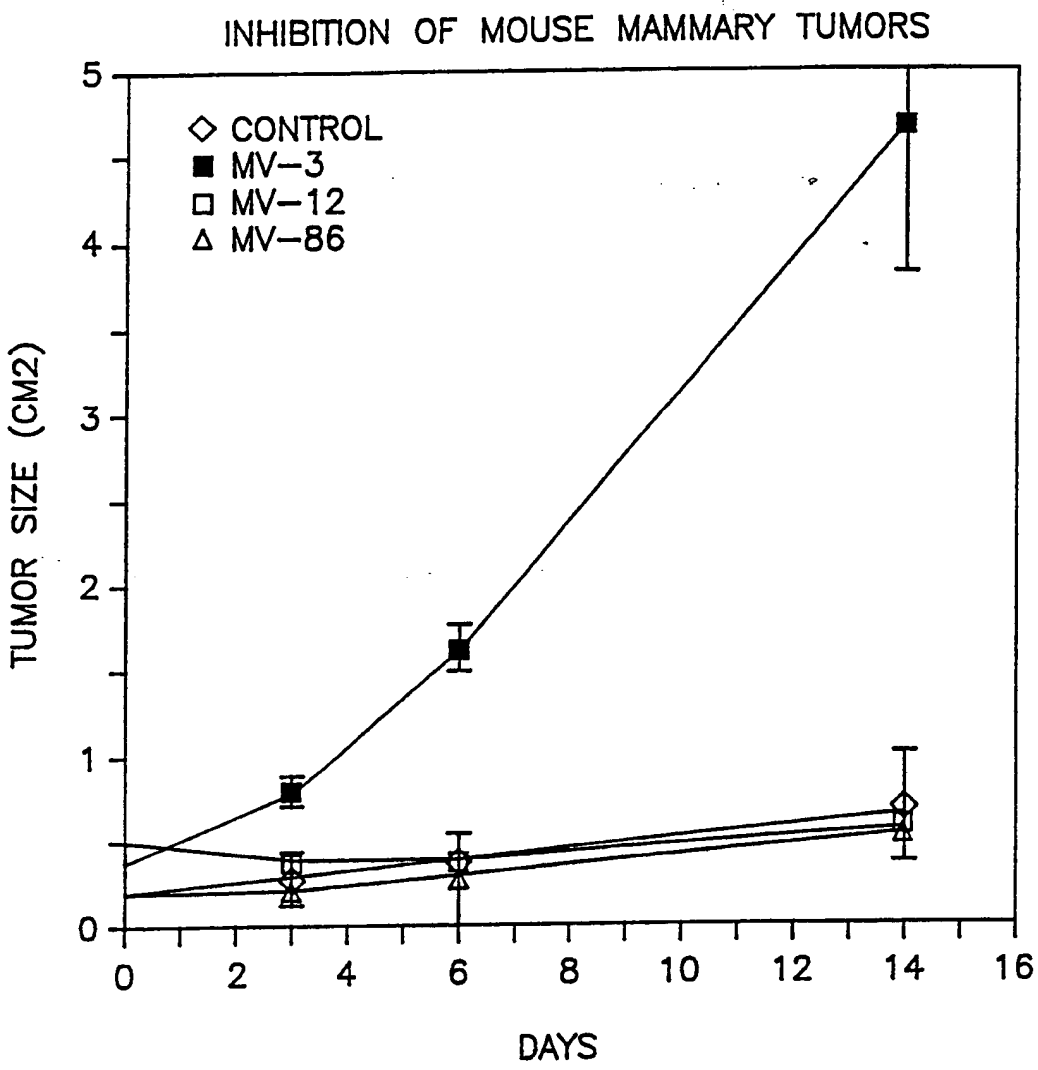

INHIBITION OF MOUSE MAMMARY TUMORS
CONTROL
MV-3
MV-12
MV-86
TUMOR SIZE (CM2)
DAYS
0
1
2
3
4
5
0
2
4
6
8
10
12
14
16

FIG. 4

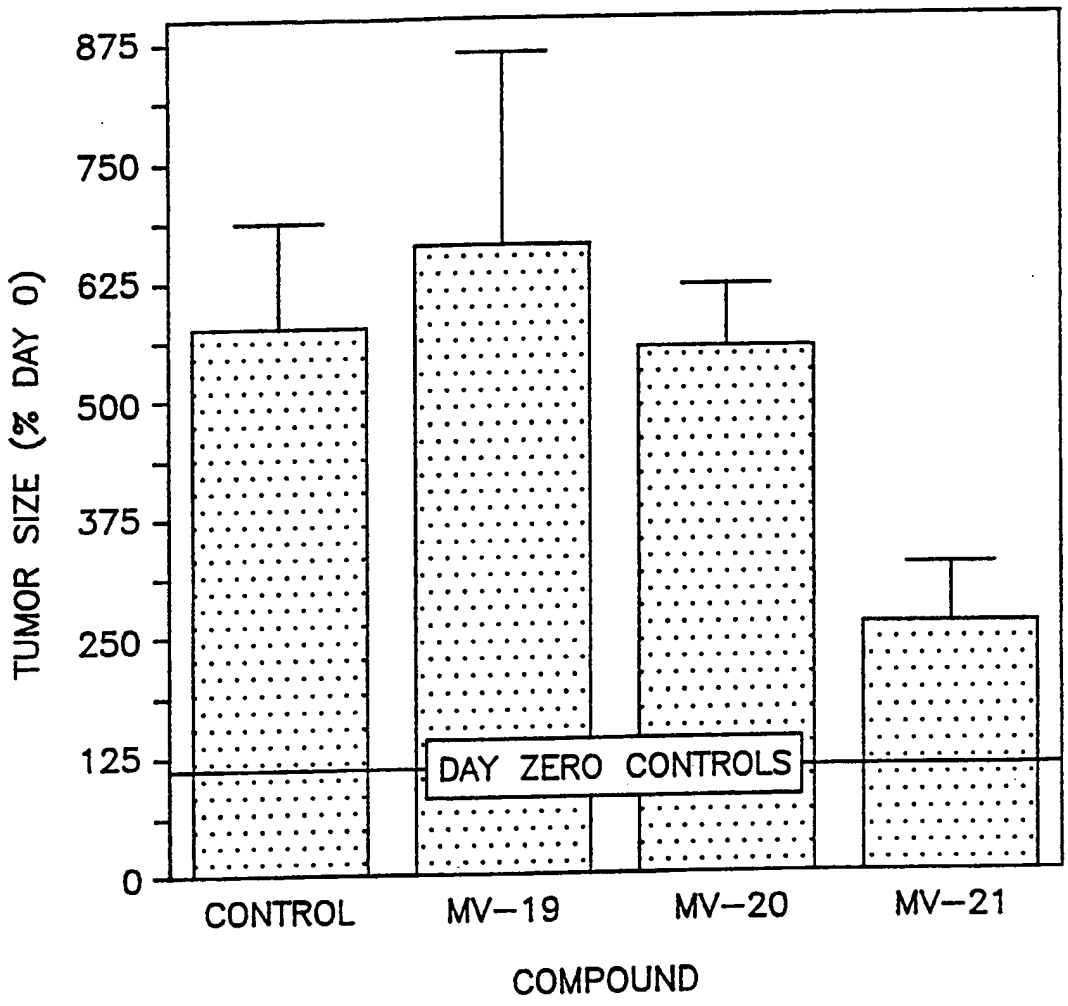

875
750
625
500
375
250
125
0
TUMOR SIZE (% DAY 0)
DAY ZERO CONTROLS
CONTROL
MV-19
MV-20
MV-21
COMPOUND

FIG. 5

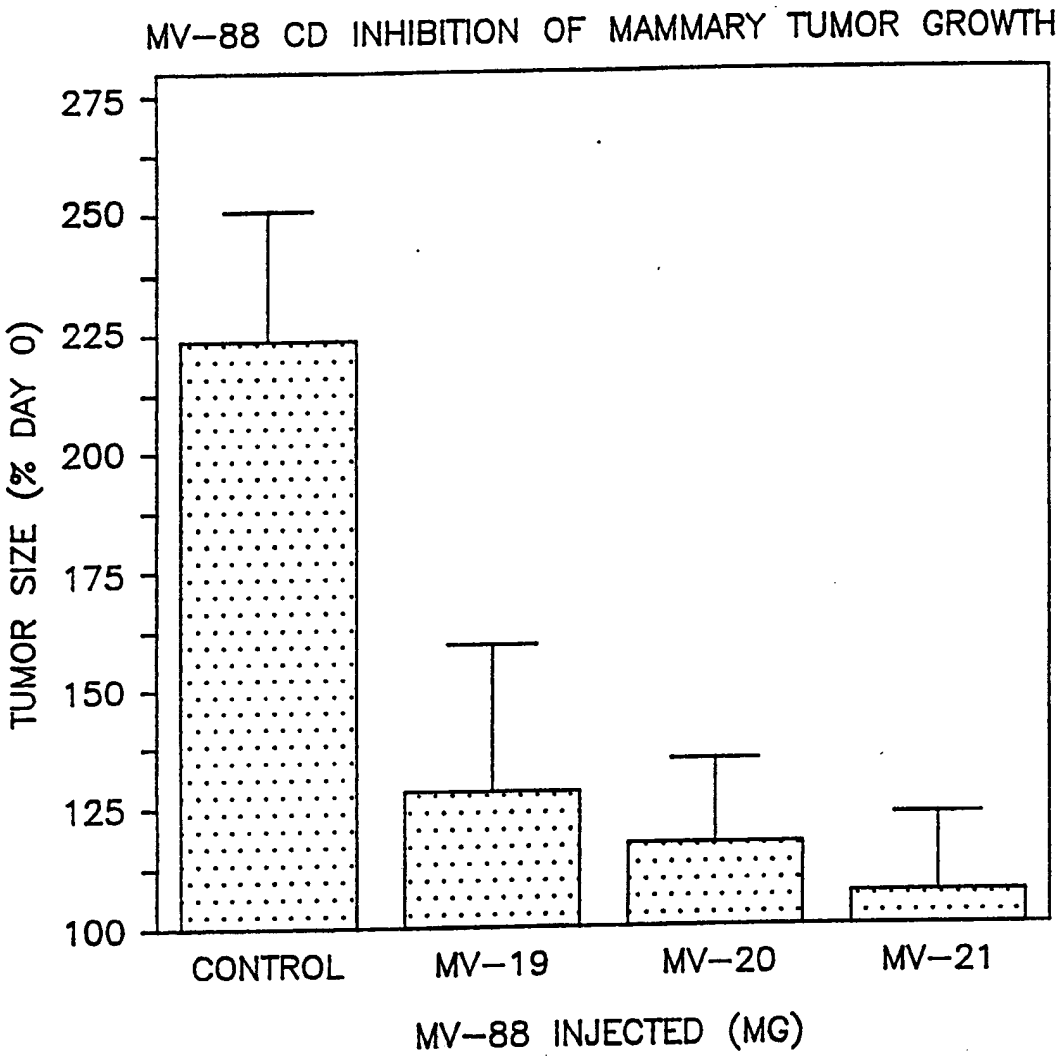

MV–88 CD INHIBITION OF MAMMARY TUMOR GROWTH
TUMOR SIZE (% DAY 0)
275
250
225
200
175
150
125
100
CONTROL
MV–19
MV–20
MV–21
MV–88 INJECTED (MG)

FIG. 6

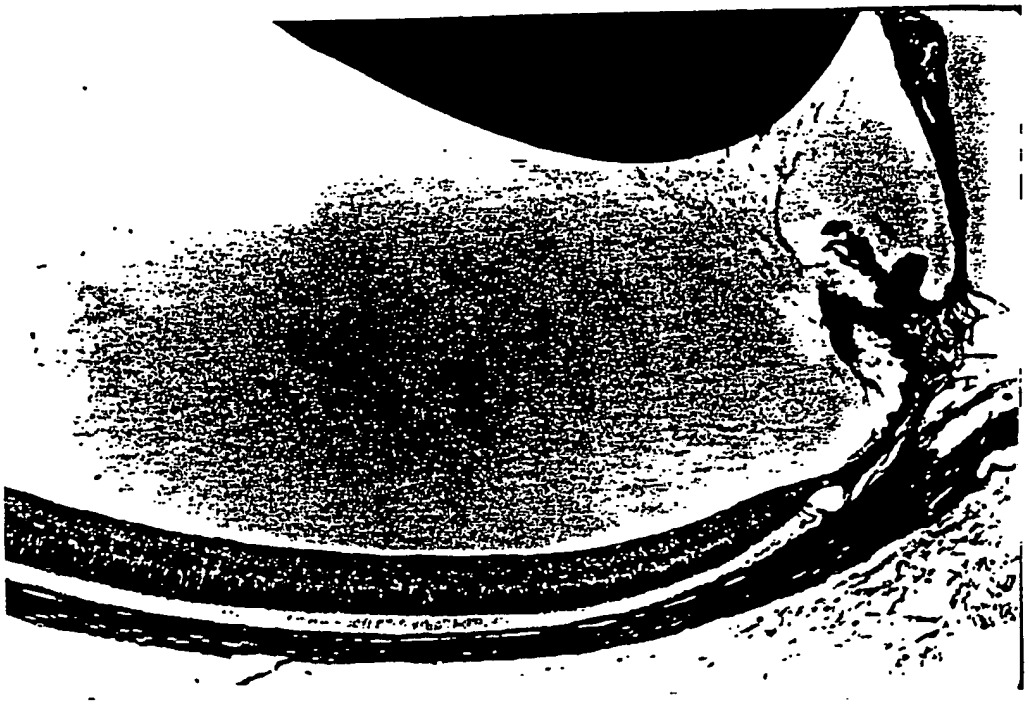


FIGURE 7a

FIGURE 7b

FIGURE 7c

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/03130

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 31/32;A61K 31/22;A61K;31/36;A61K;31/275

U.S.Cl.: 514/183;514/465;514/523;514/524;514/525/514/50/6 (see attachment)

II FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	514/183;514/465;514/523;514/524;514/525; 514/546;514/548;514/646;514/679;514/717; 514/730

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	N, Smith et al., "Mass Spectrometry of Some Substituted 2-Benzylidenecyclo Hexanones and 2, 6-Bis. Benzylidenecyclohexanones," Can. J. Chem. vol. 51, pp 1458-1470 (1973) See entire reference.	1-4, 8-12, 16,17,25-30 35,36+38-40
A	N, Edwards et al., "Chalcones, A New Class of Antimitotic Agents", J. Med. Chem., Vol. 33, 1948-1954 (1990) See entire reference.	1,7,8,15-17, 25-27,33,35 36,38-40
Y	EP, A, 0270 690 Al, Sato et al., published June 15, 1988 See entire reference	1,7,8,15-17 25-27,33,35, 36,38-40

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
06 September 1991	02 OCT 1991
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Jerome D. Goldberg

Form PCT/ISA/210 (second sheet) (Rev.11-87)